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**Effects of environmental stress on the antimicrobial drug
resistance of *Escherichia coli* of the intestinal flora of swine**

Moro, Manuel Humberto, Ph.D.

Iowa State University, 1993

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300 N. Zeeb Rd.
Ann Arbor, MI 48106

Effects of environmental stress on the antimicrobial drug resistance
of *Escherichia coli* of the intestinal flora of swine

by

Manuel Humberto Moro

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
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For the Major Department

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For the Graduate College

Iowa State University
Ames, Iowa

1993

To my wife, Ofelia, for all her dedication, support and help

..... and to the memory of my father, Manuel Moro Sommo

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GENERAL INTRODUCTION

Administration of antibiotics to animals for any purpose (growth promotion, prophylaxis or therapy) leads to the selection and accumulation of resistant bacteria in their flora (DuPont and Steele, 1987). These resistant organisms may be passed to and colonize humans, carrying R plasmids into the human environment. These R plasmids may subsequently be transferred to human pathogens or to indigenous microflora of the human body (Levy, 1992).

Gastrointestinal microflora may be disturbed by many forces including antimicrobial drugs, starvation or other dietary changes in the environment and possibly by fear and other extreme emotions (Morishita and Ogata, 1970; Holdeman et al., 1976; Moon et al., 1979; Savage, 1982; Tannock 1983). Stress of transport, overcrowding in holding pens as well as rough handling before slaughter have been reported to increase shedding of *Salmonella spp.* (Williams and Newell, 1970; Carrier et al., 1990) as well as increase the percentage of antimicrobial resistant enteric bacteria shed to the environment in pigs (Molitoris et al., 1987). Reports from a swine university herd where the prevalence of antimicrobial resistant intestinal coliforms had been decreasing progressively after discontinuing subtherapeutic feeding, mention a significant increase in antimicrobial resistance in the fecal bacterial flora following transport of these swine (Dawson et al., 1984; Langlois et al., 1986). Apparently factors other than utilization of antibiotics play a role in establishing or maintaining the antimicrobial resistant microflora of an animal.

There have been no reports on the relationship of environmental stress and its effects on the intestinal flora of swine in relationship to antimicrobial resistance.

The purpose of this investigation was to determine the effects of cold and heat stress on antimicrobial resistance of *Escherichia coli* from the intestinal tract of swine from a farm where no antimicrobials are supplemented in feed.

I. Dissertation Organization

This dissertation consists of a general introduction, a review of the literature, three separate manuscripts (PAPERS I, II, and III), a general summary, literature cited, and acknowledgements. The references cited in each manuscript are listed in REFERENCES at the end of each manuscript while the references cited in the rest of the dissertation are listed in the LITERATURE CITED at the end of the dissertation.

LITERATURE REVIEW

I. Antimicrobial Drug Use in Food Animals

Antibiotics have been used at low and subtherapeutic levels in poultry and livestock feeds for more than four decades. Nearly half of the antimicrobial agents now sold in the United States are used either therapeutically or subtherapeutically in animals. Over 31 million pounds of antibiotics are produced annually in the United States. Although accurate data on antibiotic use in animal feeds are not available, estimates indicate that almost 50% of the total annual production is directed to use in farm animals. Almost 90% of all antibiotics used in farm animals and poultry are administered in subtherapeutic concentration. About 70% of all antibiotics used in subtherapeutic concentrations in animal feed is given for the purpose of disease prevention (prophylaxis), and the remainder is administered for growth promotion (DuPont and Steele, 1987; Institute of Medicine, 1988; Frost, 1991). Tetracyclines used in livestock and poultry feeds represent almost 50% of the total of antibacterial drug use in feeds.

The use of subtherapeutic levels of antimicrobial drugs is one of the factors which has facilitated the changes of animal husbandry practices from pasture to confinement housing, allowing larger numbers of animals to be maintained in a given production facility. These changes have contributed to lowering the cost of animal care, enhancing uniformity of animal growth, increasing production of milk, eggs and meat, and ultimately lowering the cost of animal products to the consumer (DuPont

and Steele, 1987; Frost, 1991).

Certain intestinal bacteria have mildly toxemic effects on the host, which are suppressed by feed supplemented with antimicrobials. The mechanisms for the beneficial effects of antibiotics on animal growth have not been totally elucidated. There are direct effects on the gastrointestinal microflora and associated indirect effects on intestinal tissues. The direct effects include inhibition of bacterial growth, interference with bacterial cell wall development, induction of filament formation and interference with the metabolism of intestinal bacteria. Indirect effects include a reduction in the thickness of the intestinal mucosal layer and a decrease in the production of certain mucosal cell enzymes (Vissek, 1978; Walton, 1983; DuPont and Steele, 1987; Frost, 1991).

II. Risk to Humans

The use of antimicrobials at subtherapeutic levels may promote transmissible drug resistance in intestinal bacteria, especially *Escherichia coli*. Some of these resistant bacteria may be transmitted to humans, colonize the intestinal tract and cause disease (Smith, 1969; Marsik, et al., 1975; Siegel et al., 1975; Levy et al., 1976; Hirsh and Wiger, 1977; Van Houweling, 1978). Also these resistant bacteria from animals may, after colonizing the intestinal tract of humans, transfer their resistance to humans strains of Gram-negative bacteria and perhaps other microorganisms, compromising therapy of a range of human infections.

Evidence of transmission of bacteria from farm animal origin to humans has been found mainly in *E. coli* and *Salmonella spp.* (Smith, 1969; Levy et al., 1976; Hirsh

and Wiger, 1977; Hirsh et al., 1978; Threlfall et al., 1978; Hummel et al., 1986; Levy and Marshall, 1988).

In a prospective study carried out by Levy et al., (1976) evidence of increased antimicrobial resistance was observed in intestinal *E. coli* within a week after start of the feeding of tetracycline supplemented feed to a flock of chickens. Also the numbers of tetracycline resistant intestinal coliforms increased in the eleven members of this farm family, but not in their neighbors. Within 3 to 5 months after medicating the chickens, 31% of the fecal samples taken each week from each member of the farm family yielded bacterial populations of which 80% of the coliform bacteria colonies were tetracycline resistant, compared with 6.8% of the samples from neighbors. Approximately 6 months after the tetracycline was removed from the animal feed, the percentage of resistant organisms in farm dweller's fecal samples had decreased to approximately the magnitude found before use of the tetracyclines was started. In another study (Marshall et al., 1990), the potential for spread of antimicrobial resistant *E. coli* between farm animals and from farm animals to farm workers in the environment was demonstrated. In this study *E. coli* of bovine and porcine origin were marked by resistance to nalidixic acid (Na) or rifampicin (RFR) and a transferable, multiple resistant plasmid (PSL 222-1) derivative of plasmid R222 was introduced by conjugation. The two mutant derivatives were fed back to the respective host animals. This mutant persisted most of the four month period. Also these bacteria were isolated from multiple secondary hosts (pigs, fowl, flies) having direct or indirect contact with the inoculated donors. The mutants were excreted by caretakers for more than 4

weeks.

Some researchers have suggested that antimicrobial-resistant coliform organisms of farm origin may cause disease in humans (Hummel et al., 1986). They studied a pig farm in a defined territory in which the streptothricin antibiotic nourseothricin was added to pig feed to promote growth. After 2 years of use in pig feed they reported that coliform organisms containing plasmids encoding for nourseothricin resistance were present in 33% of the isolates from fecal cultures from pigs with diarrheal disease, in 18% in those from employees of the pig farms, 17% of isolates from families of employees, and in 16% of outpatients living in nearby communities. Although no nourseothricin had been used in the human population in the territory, 11% of the isolates from urinary tract infections of outpatients were nourseothricin-resistant *E. coli*. Examination of cultures from pigs, farm employees, and outpatients in neighboring territories that did not use nourseothricin in pig feed revealed no nourseothricin-resistant *E. coli*.

Antimicrobial feeding of animals may also select for resistant salmonella, with a possible increase in their quantity and prevalence. This would increase human health hazards from food poisoning strains and would be especially serious if human pathogens such as *S. typhi* and *S. enteritidis* became multiply-resistant (Institute of Medicine, 1988; Frost, 1991).

Feeding of antimicrobials to animals could compromise subsequent treatment of diseases in these animals. Also pathogenicity of bacteria might be enhanced by the selection pressure provided by continuous exposure to antimicrobials. Tissue residues

of antimicrobials might produce allergic or toxic effects in people who ingested contaminated meat or meat products (Frost, 1991).

III. Mechanism of Resistance

Introduction

Most drug resistance in clinically relevant bacteria is due to conjugative transfer of R plasmids and their clonal expansion during exposure to antimicrobial drugs. Chromosomal inheritance and the transfer of chromosomal mutant genes by transformation play a minor role (Falkow, 1975; Kreuzer, 1992).

Bacteria can acquire new genetic information in three known ways: conjugation, transduction, and transformation. Conjugation requires that the donor bacterium possess both the means to duplicate part of its genetic information and the means to attach itself to a recipient bacterium for DNA transfer. The donor must mate with a recipient bacterium that is physiologically capable of permitting the new DNA to enter and replicate autonomously as a plasmid or permitting it to become incorporated by recombination into the recipient's chromosome. Transduction is a process much less important for the transfer of drug resistance and depends on bacteriophages to "package" pieces of the chromosome or plasmid of the donor cell and inject the package into the appropriate bacterium for uptake and incorporation of the foreign DNA. Transformation is the process by which DNA in solution is taken up directly by a bacterial cell. Plasmid (circular) DNA is usually taken up more efficiently than chromosomal (linear) DNA, due to the fact that bacteria have exonucleases that attack linear fragments of DNA. Nevertheless, in some organisms, transformation of

chromosomal components has been shown to be capable of transferring drug resistance (Kreuzer, 1992).

Chromosomal Resistance

Chromosomal drug resistance, is predominantly due to mutation of pre-existing DNA. The specific sites of antibiotic binding in the target cells can occasionally be altered by mutation in such a way that an antibiotic no longer binds to the target and the target retains most of its function. Although chromosomal resistance usually involves resistance that is specific for the antimicrobial drug, it is occasionally responsible for the simultaneous appearance of resistance to several antibiotics with different structures and sites of action; e.g., the mar locus in E. coli affects uptake of tetracycline, ceftioxin, and chloramphenicol. Extensive epidemiologic investigations of drug resistance in enteric bacteria have yielded little evidence of the importance of chromosomal mutation in the acquisition of drug resistance or of transformation as a means by which drug resistance can be exchanged (Institute of Medicine, 1988). Most enteric bacteria have been shown to have low efficiency in taking up DNA (competence) unless they are treated to temporarily damage their permeability barriers.

R plasmids and transposons

Since their discovery about 35 years ago, R plasmids have been extensively studied epidemiologically and molecularly and have been shown to play a predominant role in drug resistance among bacteria (Falkow, 1975; Davies and Anandan, 1976; Davies and Smith, 1978; O'Brien et al., 1978; Davies, 1979). Like

other self replicating nonchromosomal units of DNA, R plasmids encode for efficient replication and for particular drug resistance phenotype. R plasmids carry genes that encode products that confer drug resistance in a bacterium. A single R plasmid contains multiple genes, each encoding a different kind of resistance. Some individual resistance genes encode multiple resistance to related antibacterial drugs. Almost every drug resistance determinant is carried on a genetic unit (usually small, occasionally large), called a transposon, that can move from its location on the R plasmid to other locations, typically, but not exclusively, to other plasmids. This form of DNA rearrangement, or transposition, requires special genes and sections of DNA that are parts of the transposon (Saunders, 1975; Cohen, 1976). Transposons could be considered as freely movable genetic modules that can be assorted, reassorted, and added to and subtracted from evolving R plasmids as environmental pressures dictate.

The ability of most drug resistance genes to transpose provides R plasmids with an extraordinary degree of genetic plasticity. Although it is not clear that the presence of antibiotics in the environment has any influence on the extent of transposition of resistance determinants, antibiotics exert a profound influence on the selection and persistence of R plasmids with multiple drug resistance determinants. Studies of indigenous soil bacteria in the pre-antibiotic era showed not only that R plasmids were less prevalent, but also that recovered R plasmids typically contained only one or two resistance determinants each (Davies and Amandan, 1970; Falkow, 1975). More recently, in sharp contrast, bacteria recovered in environments exposed

to antibiotics have a high prevalence of R plasmids with multiple drug resistance determinants (Finland, 1972; Falkow, 1975; O'Brien et al., 1977; 1978). It is important to note that these R plasmids are similar in many respects to the pre-antibiotic era plasmids that did not have multiple drug-resistance determinants. Transposons, whose drug resistance genes evolved as "protection" against the natural antibacterial substances in the soil, thus used plasmids already available (Falkow, 1975). Moreover, the same transposon can be found in an array of different plasmids; in nature, drug-resistance elements are promiscuous and can locate in a broad spectrum of genomes.

Many bacteria, given the appropriate supplemental genes, are potentially capable of transferring DNA to other bacteria by conjugation. Usually the supplemental genes are carried on a plasmid. When present on an R plasmid, the supplemental genes together make up what is called the resistance transfer factor, or RTF. Most R plasmids contain an RTF, which enables them to be conjugatively transferred between bacteria (Falkow, 1975). Just as the transposon, at the level of the single drug-resistance determinant, is capable of movement to a different R plasmid, the RTF-containing R plasmids are capable of movement to other strains and to other species of bacteria. Although initial studies on conjugative R plasmids were limited to facultative gram-negative bacteria (Falkow, 1975), conjugation clearly plays a major role in the transfer of drug resistance among facultative gram-positive bacteria (Smith et al., 1981; Clewell and Burke, 1986), and among anaerobic bacteria (Odelson et al., 1987). It has been found that some transposons in gram-positive bacteria can bypass

the need for RTF-containing R plasmids in conjugation (Clewell and Burke, 1986). These "conjugative transposons" contain the equivalent of the RTF, as well as the R factor. Other studies involving anaerobic bacteria have shown that sublethal concentrations of tetracycline in vitro actually promote R plasmid transfer, in addition to selecting bacteria that carry the drug-resistance determinant (Tally and Malamy, 1986).

Of equal or greater concern from the standpoint of antimicrobial drug resistance proliferation is the finding that the tetracycline resistance determinant(Tc^r) can be conjugatively transferred back and forth between *Bacteroides* (anaerobes) and *E. coli* (Tally and Malany, 1986). Inasmuch as anaerobic bacteria, especially species of *Bacteroides*, are the predominant flora in the mammalian gastrointestinal tract, the presence of back-and-forth transfer suggests that the reservoir for maintenance, persistence, and spread of at least one drug-resistance determinant, Tc^r, is enormous.

R plasmids may also contain vegetative origins of replication, or oriV, which enable them to replicate autonomously in host bacteria. Plasmids can be catalogued into "incompatibility" groups based on their oriV; those with the same oriV cannot coexist within a bacterium, because of competition for identical replication factors (Falkow, 1975). The specific oriV carried by a plasmid constitutes another element of its phenotype: its host range. Narrow host range plasmids can replicate only in few species of bacteria, usually because only those bacteria provide additional factors required for plasmid replication. Broad-host-range plasmids can transfer to and replicate in a large variety of bacteria. Results of transfer studies in *E. coli* and

Bacteroides indicate that antibiotic exposure can increase the transfer and selection of at least one form of broad-host-range plasmid resistance to tetracycline.

In early studies of conjugative transfer of R plasmids among bacteria common in the gastrointestinal tracts of humans and farm animals, *E. coli* was found to act as a good donor and recipient of R plasmids; *Salmonella spp.* were not as good donors and recipients (Falkow, 1975). In a more recent study involving *Salmonella typhimurium* and *E. coli* recovered from calves, strains of *S. typhimurium* were identified which are extremely proficient R plasmid donors, even better than strains of *E. coli* (Timoney, 1981). Certain plasmids belonging to a particular group of incompatible plasmids, H2, found in most strains of salmonellae and preferentially in *S. typhimurium*, showed a peak efficiency of transfer at 30°C and were conjugatively transferred in calves' feces after excretion (Timoney, 1981). H2 plasmids have other critical features: they carry resistance to both penicillin and tetracycline, they transfer to *E. coli*, and they carry other non-drug-resistance determinants that increase the ability of the bacteria to colonize the gastrointestinal tract (Timoney, 1981). In every general aspect of drug resistance studied (i.e., expression of resistance genes, maintenance of R plasmids, and transfer of plasmids), *Salmonella spp.* and *E. coli* have been found to be quite similar (Falkow, 1975). Although *Salmonella spp.* might behave quite differently from *E. coli* in the gastrointestinal tract, because of the former organisms' ability to invade enterocytes and thereby avoid antibiotics that cannot efficiently penetrate the cell (i. e., penicillin and aminoglycosides but not the tetracyclines or chloramphenicol), evidence suggests that R plasmid transfer may

occur with ease in *Salmonella* spp. in vivo. In an outbreak of gastroenteritis caused by *S. typhimurium* that affected 1,900 persons who ate contaminated turkey meat, the source strain of bacteria isolated from the meat was antibiotic susceptible, as were bacterial organisms isolated from persons who had not taken any antibiotics. In sharp contrast, a high proportion of the persons who were given ampicillin, chloramphenicol, or one of several other antibiotics had *S. typhimurium* with R plasmids encoding for resistance to such antibiotics in their stools; this proves the ability of salmonellae to acquire R plasmids from the human gut (Aserkoff and Bennett, 1969). In this way, strains of salmonella, as well as of *E. coli*, can act as reservoirs for conjugative R plasmids and thus gain an enormous selective advantage over other bacteria when antibiotics are present in the environment.

Epidemiological surveys of R plasmids in clinically important bacteria have shown that multiple drug resistance has increased progressively since the beginning of the antibiotic era (Finland, 1972; O'Brien et al., 1977; 1978). Among individual R plasmids, the number of drug resistance genes per R plasmid and the likelihood that a given R plasmid is conjugative have also been increasing (Neu et al., 1975; Levy, 1978; Smith et al., 1981). Although early studies had indicated that the problem of drug resistance was most pronounced in the Enterobacteriaceae, the more recent studies show that the problem has spread. Drug resistance has now been found in bacteria of nearly all genera that are important (O'Brien et al., 1978; Davies, 1979; Finland, 1979; Lacey, 1979; Smith et al., 1981; Clewell and Burke, 1986; Tenover, 1986; Tally and Malamy, 1986; Odelson et al., 1987).

IV. Effects of stress, diet, and environment on the stability of the gastrointestinal microflora

Gastrointestinal microflora are known to be important to the health and welfare of their animal hosts (Savage, 1982). Microflora function optimally when they are composed of particular microbial species functioning in specific niches localized in particular habitats in the stomachs or intestines (Savage, 1982, Tannock, 1983). Numerous complex factors influence directly what species can occupy niches available in the gastrointestinal tract.

Microflora may be disturbed by many factors including antimicrobial drugs, starvation, dietary changes, alterations in the environment, and possibly by fear and other extreme emotions (Morishita and Ogata, 1970; Holdeman et al., 1973; Moon et al., 1979; Tannock, 1983).

Adverse dietary and environmental conditions are said to "stress" animals. The term stress is commonly used to denote pressing external demands on an animal. In humans; stress may denote an emotional state in which the subject perceives that his /her response is inadequate to cope with a difficult or new situation.

The anterior lobe of the pituitary gland produces, stores and releases adrenocorticotrophic hormone (ACTH). This substance stimulates the adrenal cortex to synthesize corticoids, which are released into the blood. Numerous factors can contribute to lead to the release of ACTH by the pituitary gland such as certain chemicals, heat, cold, sound, light, atmospheric pressure, fear, pain, anxiety, anger, frustration, fatigue, surgery (trauma), ether anesthesia, long airplane flights, hypoxia

plus heat, electrical shock, diabetes mellitus, insulin, acute illness, glucagon, pyrogens, pregnancy, estrogen treatment, starvation, salicylates, trained fighting behavior in mice and animal management practices on farm (Safran, 1962; Yates and Urquhart, 1962; Kilgour and Langen, 1970; Dvorack, 1971; Brain, 1972; Tannock, 1983). The activation of the pituitary - adrenal system by stress serves to promote metabolic responses that help animals to face the altered conditions. The most important metabolic effect is probably that imposed on carbohydrate and protein metabolism. The glucocorticoids released by the adrenals influence the amount of glycogen deposited in the liver, so that blood sugar levels are maintained. Stress also stimulates the release of epinephrine from the adrenal medulla and the secretion of hormones from the posterior pituitary. These substances influence smooth muscle, the secretion of gonadotropins, and the regulation of electrolytes and body fluid (Safran, 1962; Tannock, 1983).

Starvation and abrupt changes in the diet could alter the gastrointestinal ecosystem in several ways. For example they have been shown to decrease the desquamation rate of the jejunal epithelium of cats (Goldsmith, 1973) and mice (Komai and Kimura, 1979). In ruminants, the number of ruminal microbes decreased in starvation (Hungate, 1966; Grubb and Dehority, 1975). In monogastric animals starvation and environmental stress produced a reduction in the number of lactobacilli in the stomach and small intestine (Smith, 1965; Tannock and Savage, 1974). The population of fusiform - shaped bacteria associated with the mucosal epithelium of the large intestine is lower in stressed mice than in nonstressed animals. The number of

coliforms was higher in the lower small intestine and in the large intestine of stressed mice than in those of control animals. Pigs deprived of food for 24 hours or food and water for 72 hours had decreased numbers of lactobacilli and bifidobacteria in the stomach and proximal jejunum. *E. coli* and *Bacteroides* numbers increased in the ileum of the stressed animals (Morishita and Ogata, 1970). In several investigations made on children suffering from protein-calorie malnutrition (Mata et al., 1972; Gracey et al., 1973; Heyworth and Brow, 1975) it was shown that coliform numbers increased under conditions of dietary stress for the host.

The number of bacterial coliforms are regulated principally by the anaerobic flora of the large intestine. Volatile fatty acids produced in the large intestine by anaerobic bacteria inhibit the growth of coliform bacteria (Schaedler, 1965; Lee and Gemmel, 1972). Dietary stress, which alters the gastrointestinal ecosystem, presumably influences the anaerobes inhabiting that site. Any decrease in the metabolic activities of these microbes will therefore be reflected in a rise in the number of coliforms.

Stress of transport, overcrowding in holding pens and rough handling before slaughter has been shown to increase the shedding of certain enteric bacteria like salmonellae (William and Newell, 1970). A 30% increase in *Salmonella* spp. shedding was reported after pigs were loaded and transported to a slaughterhouse. In a study on the effects of fecal excretion of salmonellae in calves, an increase in *Salmonella* spp. isolation was observed from 0% pre - stress to 8% after stress (Corrier et al., 1990).

Changes in antimicrobial resistance in fecal bacteria associated with transport and holding stress at slaughterhouses in hogs have been reported (Molitoris et al., 1987). Moreover, when pigs from an antibiotic-free herd were transported over 322 km. there was a significant increase in the level of resistance to sulfoxazole, streptomycin and tetracycline (Langlois et al., 1984).

Fecal streptococci from hogs on farms and after transport to a slaughterhouse showed an increase in the percentage of resistance to cephalothin, chloramphenicol, erythromycin, lincomycin, methicillin, neomycin, penicillin, streptomycin, sulfadiazine, and tetracycline (Molitoris et al., 1987). Resistance to chloramphenicol, erythromycin, lincomycin, methicillin, neomycin, streptomycin, and tetracycline increased significantly in coliform bacteria after long holding times (43 hours) in hogs at slaughterhouses (Molitoris et al., 1987). The stress associated with moving hogs from one location to another increased both the levels of antibiotic resistance and the incidence of multiple resistance (Langlois et al., 1984; Dawson et al., 1984). Also, after antimicrobial resistance in fecal coliforms was increased due to stress of moving or changing environment, a relatively long period of time was required for the resistance level to decrease significantly (Dawson et al., 1984). It seems that factors other than feeding or use of antibiotics play also a role in establishing or maintaining the antibiotic resistant microflora of animals (Langlois et al., 1984).

**PAPER I. EFFECTS OF COLD STRESS ON THE ANTIMICROBIAL DRUG
RESISTANCE OF *ESCHERICHIA COLI* OF THE INTESTINAL FLORA
OF SWINE**

ABSTRACT

The effects of cold stress on the antimicrobial drug resistance of intestinal *Escherichia coli* from swine on a farm where no antimicrobials were added to feed for 10 years was studied. Fecal samples were initially collected from animals of different age groups(growers, finishers, gilts, and sows). Subsequently finishers were sampled over a period of 2 years. Samples were collected over periods considered seasonally normal and stable (baseline) as well as during times in which drastic drops in environmental temperature (cold stress) occurred. Baseline bacterial resistance levels of prevalence were significantly higher ($P < 0.05$) in younger pigs than older pigs to ampicillin and tetracycline. Also when animals were exposed to excessively cold conditions, there was a significant ($P < 0.05$) increase in ampicillin and tetracycline resistance in *E. coli* for animals of all age groups.

INTRODUCTION

It is well recognized that the administration of antibiotics to animals for any purpose (growth promotion, prophylaxis or therapy) leads to the accumulation of resistant bacteria in their flora (DuPont and Steele, 1987). The danger of this to humans is that: 1) Antibiotic - resistant pathogens common to animals and humans may reach the latter by cross infection, and 2) Antibiotic resistant non-pathogenic organisms in an animal may be passed to and colonize humans, carrying R plasmids into the human environment. These R plasmids may subsequently be transferred to human pathogens or to indigenous flora in the human body (Levy, 1992).

In swine, stress of transport, overcrowding in holding pens as well as rough handling before slaughter has been reported to increase shedding of *Salmonella spp.* (Williams and Newell, 1970; Corrier et al., 1990) as well as to increase the percentage of antimicrobial resistant enteric bacteria shed to the environment in pigs (Molitoris et al., 1987). Reports from a university swine herd, in which the prevalence of antimicrobial drug resistant *E. coli* decreased progressively after 13 years of discontinuing subtherapeutic feeding, show a significant increase in antimicrobial resistance following transport of these animals (Dawson et al., 1984; Langlois et al., 1986).

There have been no reports on the relationship of environmental stress on intestinal microflora of swine to antimicrobial resistance.

The effects of cold stress on the prevalence of antimicrobial resistance in *E. coli*

from the intestinal tract of swine from a farm where no antimicrobials are supplemented in feed were examined to determine changes associated with environmental (cold) stress.

MATERIALS AND METHODS

Animals, Housing, and Management

The study reported in this paper was carried out at a farm where no antimicrobials had been incorporated into swine feed for the past ten years. Therapeutic use of antimicrobial drugs has been limited to very rare injections of penicillin, streptomycin, or tylosin. This operation sells approximately 1000 finisher pigs per year. It is a closed herd with approximately 80 gilts and sows. Animals are maintained in semiconfinement and they have partial protection against extreme weather conditions. Feed (corn and soybean meal) is produced and mixed on the premises. Management practices have been maintained constant for the past years.

During the past 2 years, fresh fecal samples (1 per animal) were collected monthly in order to monitor the prevalence of antibiotic resistant *E. coli* and a profile of antibiograms of the herd was obtained. Initially 10 randomly selected pigs from each of the following age groups were sampled: (a) multiparous sows; (b) gilts in first gestation; (c) grower pigs between 6 and 10 weeks old; (d) finisher hogs between 4 and 5 months of age. In subsequent fecal collections only 10 randomly selected finisher hogs were sampled. Collections were made following a period of at least 7 days of weather considered seasonally normal and stable (baseline). Cold stress fecal samples were collected 24 hours following sudden and drastic drops in temperature ($> 15^{\circ}\text{C}$) during winter months.

Microbial Analysis

Ten-fold serial dilutions of sterile buffered saline suspensions of 5 g fecal material were plated on Tergitol¹ 7 agar with TTC (triphenyltetrazolium chloride). Plates were incubated at 37 °C for 18-24 hours. Ten smooth *E. coli* colonies were randomly picked from each culture and biochemically identified (Edwards and Ewing, 1986).

Antimicrobial susceptibility results were determined using a broth dilution breakpoint method in which the antimicrobials were prepared commercially in a microliter plate format². Tested antimicrobials and breakpoints are shown in Table 1.

After initial broad spectrum screening for resistant bacteria, ampicillin and tetracycline were the antimicrobials selected. In subsequent samples an agar dilution procedure was utilized with breakpoints at 8 µg/ml for ampicillin and 4 µg/ml for tetracycline³ (Lorian, 1986; Sahm and Washington, 1991; Koneman et al., 1992).

Counts for that portion of total coliform population (lactose positive enteric bacteria) resistant to ampicillin and tetracycline were determined by plating in duplicate ten-fold dilutions of intestinal contents as described above on MacConkey agar and MacConkey agar plus 25 µg ampicillin/ml or 25 µg tetracycline/ml. Plates were incubated at 37 °C for 24 h and lactose positive colonies were counted. The

¹ Difco Manual, Difco Laboratories, Detroit, MI.

² SENSITITRE Ltd, The Manor Royal, Crawley, West Sussex, RH10 2PY, England.

³ Sigma Chemical Co., St. Louis, MO.

percentage of the coliform population resistant to ampicillin and tetracycline was determined by dividing the count obtained on MacConkey agar plus 25 μ g ampicillin/ml and 25 μ g tetracycline/ml by the count obtained on MacConkey agar without antibiotics and multiplying by 100 (Langlois et al., 1978).

Statistical Analysis

Mean coliform counts were subject to log transformation before statistical analysis. The data were analyzed by ANOVA. Differences were considered significant at $P < 0.05$ level (Snedecor and Cochran, 1989).

Table 1. Antimicrobial Agents Used for Susceptibility Testing

Antimicrobial agent	Concentration Range $\mu\text{g/ml}$
Amikacin	4, 32
Ampicillin	1, 2, 8, 16
Apramycin	16, 64
Augmentin	1/0.5, 16/8
Ceftiofur	2, 4
Cephalothin	4, 8
Clindamycin	0.5, 4
Erythromycin	1, 4
Gentamicin	2, 4, 8, 16
Methicillin + 2% NaCl	8
Spectinomycin	8, 16, 32, 64
Penicillin	0.03, 1, 16
Sulphachloropyridazine	100, 200
Sulphadimethoxine	100, 200
Tetracycline	1, 4, 16
Tribiassin	0.5/9.5, 2/38
Tylosin Tartrate	5, 10, 20

RESULTS

Initial screening with Sensititre plates showed significant changes in resistance to ampicillin and tetracycline by age group and by exposure or nonexposure to cold stress. Percentage of resistance by age group as well as per stress exposure are shown in Tables 2 and 3 and by antibiotic resistance in Figures 1 and 2.

Baseline prevalence of resistance for the period of the study (April 1991-June 1993) ranged from 5% to 10% for ampicillin and from 25% to 55% for tetracycline. Prevalence of ampicillin resistance under "cold stress" increased up to 38% and was statistically significant ($P < 0.05$) (Figure 3).

Tetracycline resistance prevalence ranged from 67% to 80% and was significantly higher than baseline prevalence ($P < 0.5$), (Figure 4).

Antimicrobial resistance patterns are shown in Table 4. The cold stress group showed an increase mainly in the incidence of the ampicillin + tetracycline pattern.

Prevalence of ampicillin and tetracycline resistance in total coliforms was similar to that obtained for *E. coli*.

Table 2. Antimicrobial Resistance of *E. coli* isolated from Pigs of Different Ages

Baseline prevalence

<u>Antimicrobial Agent</u>	<u>Age Group</u>			
	Growers	Finishers	Gilts	Sows
Ampicillin	20%	12%	9%	8%
Tetracycline	68%	55%	42%	51%

Table 3. Antimicrobial Resistance of *E. coli* isolated from Pigs of Different Ages

Stress prevalence

<u>Antimicrobial Agent</u>	<u>Age Group</u>			
	Growers	Finishers	Gilts	Sows
Ampicillin	55%	35%	40%	45%
Tetracycline	90%	75%	65%	75%

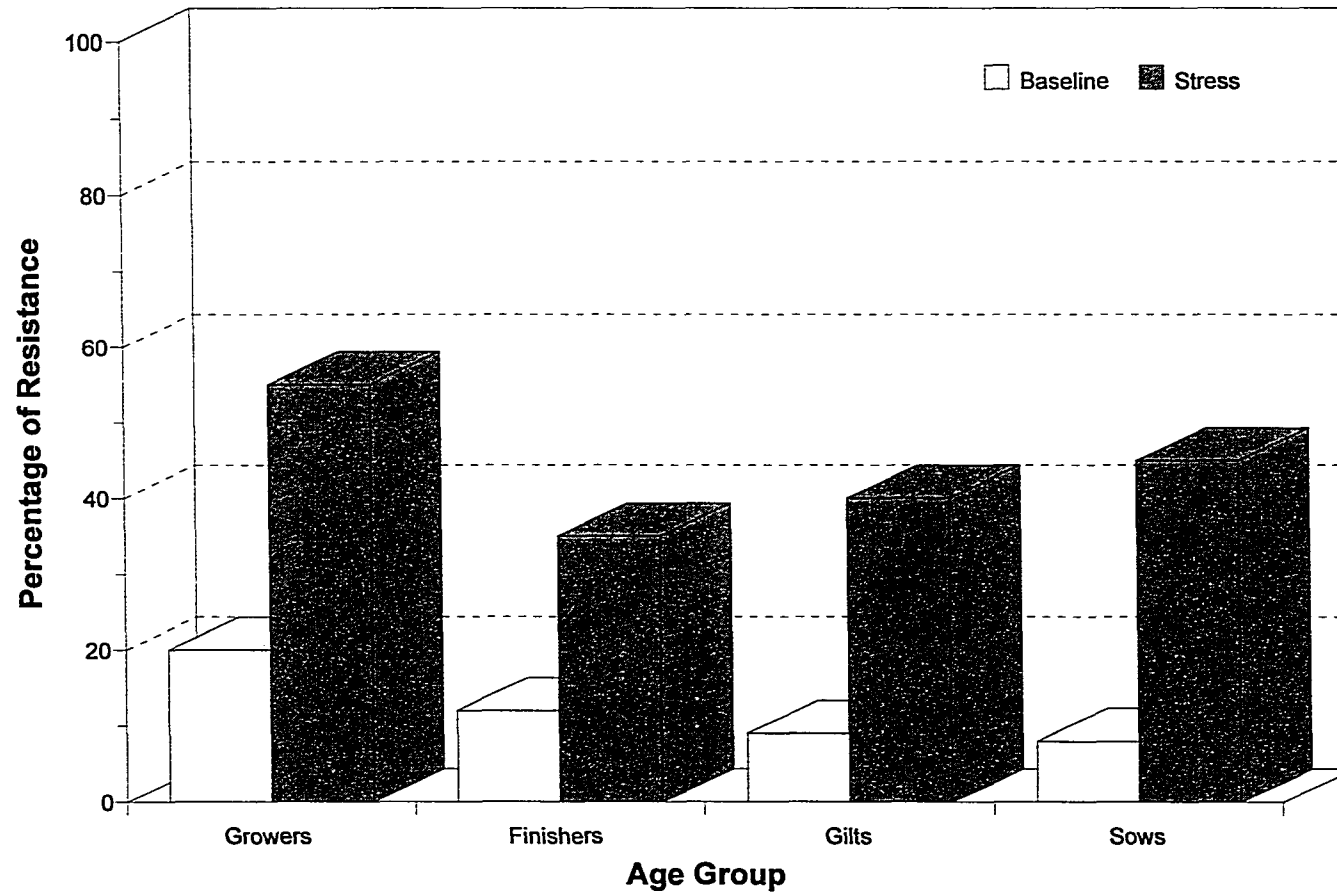


Figure 1. Baseline and Cold Stress Levels of Ampicillin Resistance for Different Ages of Pigs

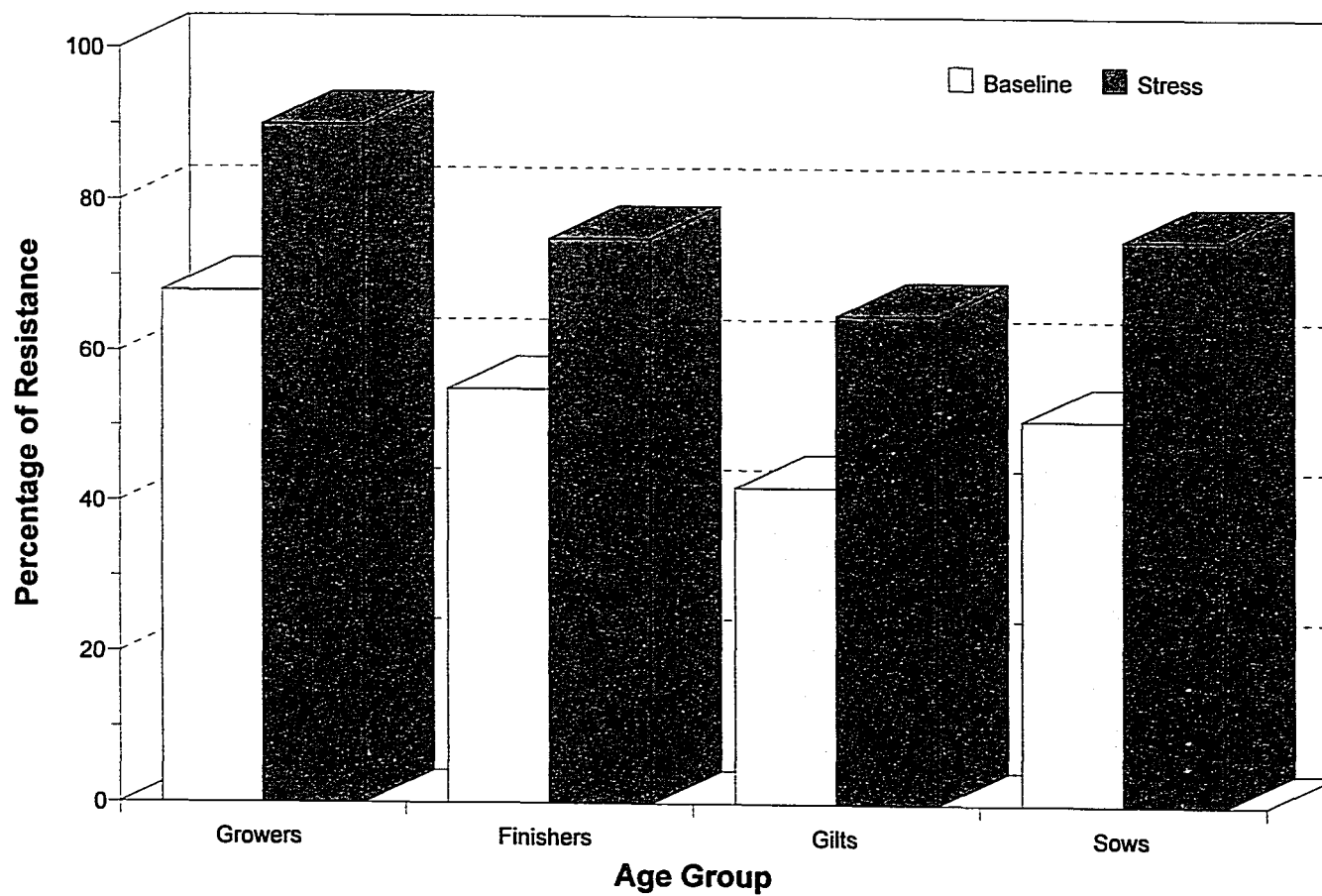


Figure 2. Baseline and Cold Stress Levels of Tetracycline Resistance for Different Ages of Pigs

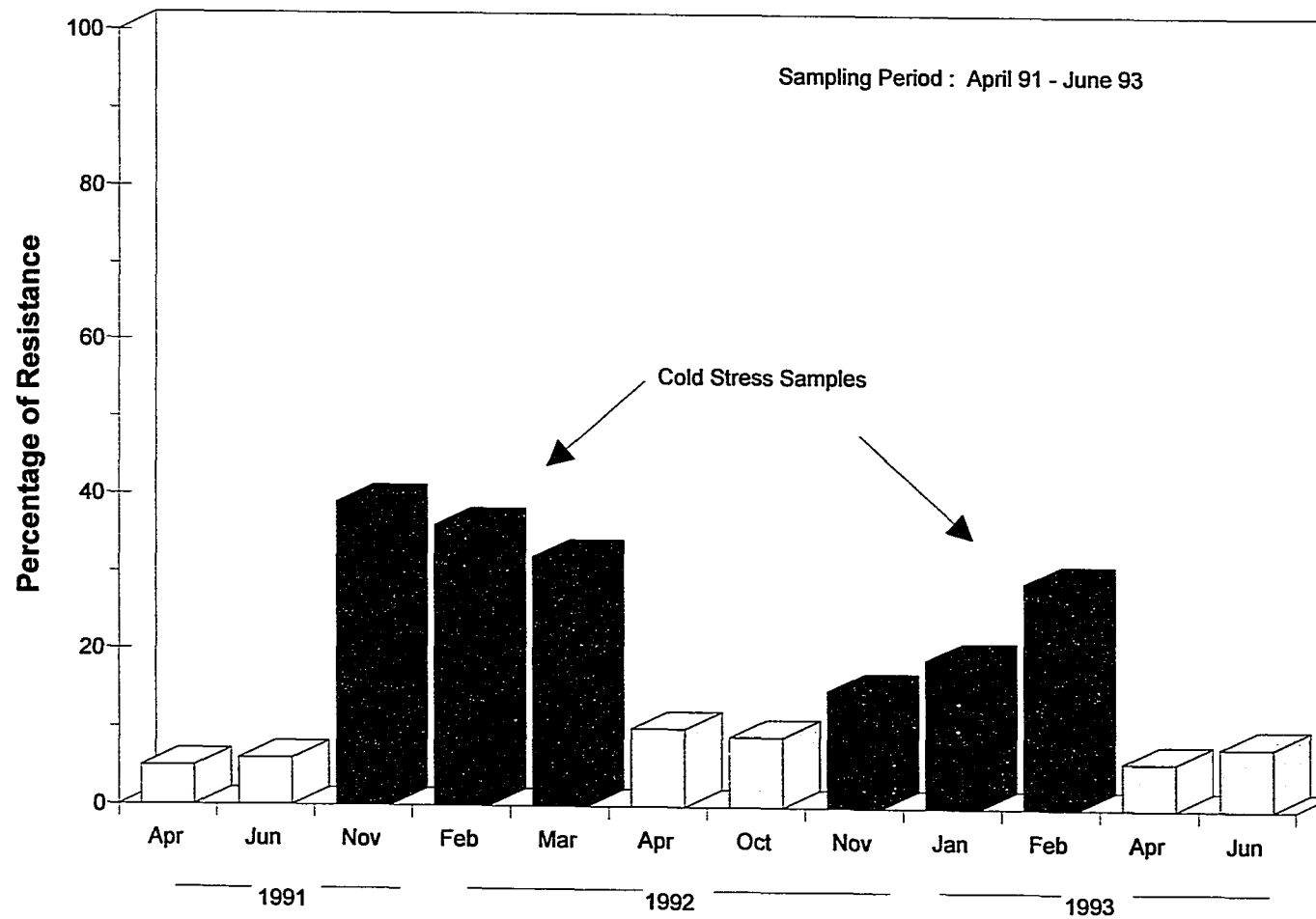


Figure 3. Effects of Cold Stress. Ampicillin Resistance of *Escherichia coli*.

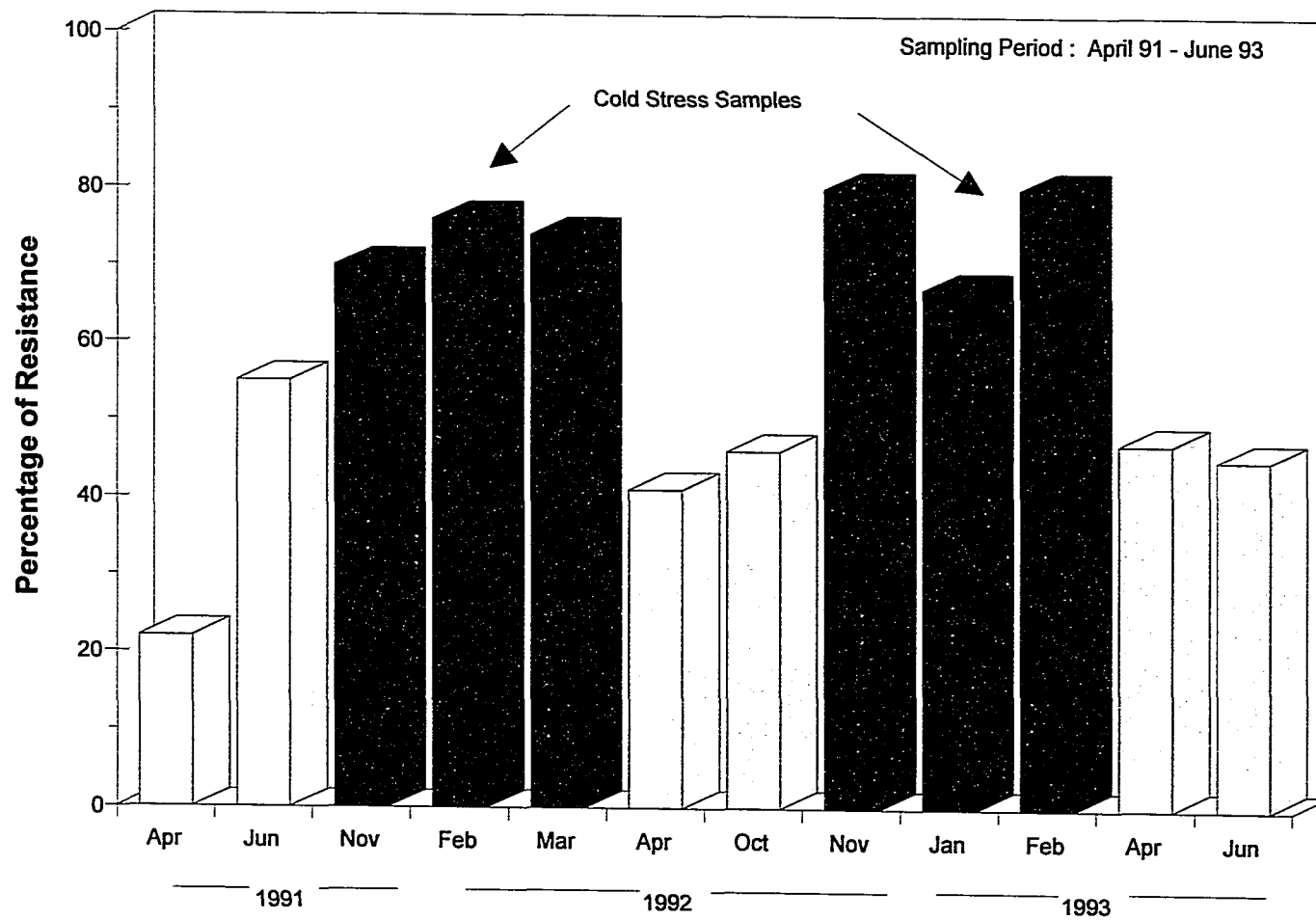


Figure 4. Effects of Cold Stress. Tetracycline Resistance of *Echerichia coli*.

Table 4. Antimicrobial Resistance Patterns for *E. coli* Isolated from Pigs

Resistance Pattern	Baseline (493) ^a	Cold Stress (472)
0	48%	23%
Ampicillin	3%	4%
Tetracycline	43%	46%
Ampicillin + Tetracycline	6%	27%

^aNumber of isolates examined.

DISCUSSION

Baseline prevalence of resistance was higher in *E. coli* from younger animals (growers) than from animals older than 4 months of age (finishers, gilts, and sows). Antimicrobials were not used in the swine of any ages. The relationship of age and antibiotic resistance of *E. coli* from the intestinal tract is difficult to explain since young pigs have been exposed to the microflora of their dams. Other investigators, however; (Guinee, 1972; Linton et al; 1972, 1975; Sogaard; 1973; Wierup, 1975; Hinton et al., 1985, 1986) have reported higher levels of resistance and greater incidence of multiple resistance in young animals and in children than in older animals and adults. The relationship of age and antibiotic resistance may suggest that the gastrointestinal tract in younger animals may be colonized more readily than in older animals by antibiotic resistant organisms in the absence of any antibiotic selection pressure (Hartley and Richmond, 1975; Petrocheilou et al., 1976, 1977). This will suggest that the survival of these strains is dependent on the characteristics of the strains other than the resistance genes (Hinton, 1986).

Swine can survive over a wide range of environmental temperatures but their health and productivity performance vary considerable within this range. Swine become more cold resistant as they grow; although all cold stress samples collected during this study were well below the lower border of the zone of thermoneutrality (lower critical temperature) for swine which has been reported as 10 °C for finisher pigs (Young, 1981; Curtis, 1984).

Measurement and interpretation of "natural" environmental stresses on animals may be a difficult task as numerous factors are acting in concert. Naturally occurring cold stress caused statistically significant increases in the prevalence of ampicillin and tetracycline resistance of *E. coli* nearly every time the temperature decreased.

Environmental stressors may influence nutrient requirements and productivity of animals by affecting heat exchange and rate of feed intake. This could affect the gastrointestinal microflora by altering and/or favoring the multiplication of certain anaerobic species. Moreover such increase of certain anaerobic species of bacteria can subsequently change the pH, oxidation-reduction potential, production of hydrogen peroxide, etc., which can deter the growth of some bacterial species in favor of other types as aerobic *E. coli* (Mitsuoka, 1978; Savage, 1982; Tannock, 1983).

This study has shown that factors other than the exposure to subtherapeutic or therapeutic antimicrobials play a role in enhancing the resistant *E. coli* populations in swine intestines.

REFERENCES

- Barry, A. L.** 1986. Procedure for testing antimicrobial agents in agar media: theoretical considerations, p. 1-26. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 2nd ed. Williams & Wilkins Co., Baltimore.
- Corrier, D. E., C. W. Purdy, and J. R. DeLoach.** 1990. Effects of marketing stress on fecal excretion of *Salmonella spp* in feeder calves. *Am. J. Vet. Res.* **51**: 866-869.
- Curtis, S. E.** 1985. Physiological responses and adaptations of swine, p. 129-148. *In* M. K. Yousef (ed.), Stress physiology in livestock. Vol II Ungulates. CRC Press, Inc., Boca Raton.
- Dawson K. L., B. E. Langlois, T. S. Stahly, and G. L. Cromwell.** 1984. Antibiotic Resistance in anaerobic bacteria from the intestinal tract of swine fed therapeutic and subtherapeutic concentration of chlortetracycline. *J. Anim. Sci.* **58**: 123-131.
- Dupont, H. L., and J. H. Steele.** 1987. Use of antimicrobial agents in animal feeds: implications for human health. *Rev. Infect. Dis.* **9**: 447-460.
- Ewing, W. H.** 1986. Edwards and Ewing's Identification of Enterobacteriaceae. 4th edition. Elsevier Science Publishing Co., Inc., New York.
- Gulnee, P.A.** 1972. Bacterial drug resistance in animals, p 95-104. *In* V. Kremery, L. Rosival, and T. Watanabe (ed.), Bacterial plasmids and antibiotic resistance. Springer-Verlag, New York.
- Hartley, C. L., and M. H. Richmond.** 1975. Antibiotic resistance and survival of *E. coli* in the alimentary tract. *Br. Med. J.* **4**: 71-74.
- Hinton, M., D. J. Hampson, E. Hampson, and A. H. Linton.** 1985. A comparison of the ecology of *Escherichia coli* in the intestine of healthy unweaned pigs and pigs after weaning. *J. Appl. Bacteriol.* **58**: 471-478.
- Hinton, M., A. H. Linton, and A. J. Hedges.** 1985. The ecology of *Escherichia coli* in calves reared as dairy-cow replacements. *J. Appl. Bacteriol.* **58**: 131-138.
- Hinton, M.** 1986. The ecology of *E. coli* in animals including man with particular reference to drug resistance. *Vet. Rec.* **119**: 420-426.
- Hinton, M., A. J. Hedges, and A. H. Linton.** 1985. The ecology of *Escherichia coli* in market calves fed a milk-substitute diet. *J. Appl. Bacteriol.* **58**: 27-35.

Koneman, E. W., S. D. Allen, W. M. Janda, P. C. Schreckenberger, and W. C. Winn, Jr. 1992. Antimicrobial susceptibility testing, p. 630-631. *In* E. W. Koneman, S. D. Allen, W. M. Janda, P. C. Schreckenberger, and W. C. Winn, Jr. (ed.), Color atlas and textbook of diagnostic microbiology, 4th ed. J. B. Lippincott Co., Philadelphia.

Langlois, B. E., K. A. Dawson, G. L. Cromwell, and T. S. Stahly. 1986. Antibiotic resistance in pigs following a 13 year ban. *J. Anim. Sci.* **62**: 18-32 (Suppl. 3).

Langlois, B. E., G. L. Cromwell, and V. W. Hays. 1978. Influence of chlortetracycline in swine feed on reproductive performance and on incidence and persistence of antibiotic resistant enteric bacteria. *J. Anim. Sci.* **46**: 1369-1382.

Levy, S. B. 1992. The antibiotic paradox: how miracle drugs are destroying the miracle. Plenum Press. New York.

Linton, K. B., K. Howe, and A. D. Osborne. 1975. The effects of feeding tetracycline, nitrovin and quindoxin on the drug resistance of coli-aerogenes bacteria from calves and pigs. *J. Appl. Bacteriol.* **38**: 255-275.

Linton, K. B., P. A. Lee, M. H. Richmond, W. A. Gillespie, A. J. Rowland, and V. N. Baker. 1972. Antibiotic resistance and transmissible R-factors in the intestinal coliform flora of healthy adults and children in an urban and a rural community. *J. Hyg.* **70**: 99-104.

Linton, A. H., B. Handley, and A. D. Osborne. 1978. Fluctuations in *Escherichia coli* O-serotypes in pigs throughout life in the presence and absence of antibiotic treatment. *J. Appl. Bacteriol.* **44**: 285-298.

Mitsuoka, T. 1978. Intestinal bacteria and health, p. 98-108. Harcourt Brace Jovanovich, Tokyo.

Mollitoris, E. D. J. Fagerberg, C. L. Quarles, and M. I. Krichevsky. 1987. Changes in antimicrobial resistance in fecal bacteria associated with pig transit and holding times at slaughter plants. *Appl. Environ. Microbiol.* **53**: 1307-1310.

Petrochellou, V., J. Grinsted, and M. H. Richmond. 1976. R-plasmid transfer *in vivo* in the absence of antibiotic selection pressure. *Antimicrob. Agents Chemother.* **10**: 753-761.

Petrochellou, V., and M. H. Richmond. 1976. Distribution of R plasmids among the O-antigen types of *Escherichia coli* isolated from various clinical sources. *Antimicrob. Agents Chemother.* **9**: 1-5.

Petrochellou, V., M. H. Richmond, and P. M. Bennett. 1977. Spread of a single plasmid clone to an untreated individual from a person receiving prolonged tetracycline therapy. *Antimicrob. Agents Chemother.* **12**: 219-225.

Sahn, D. F., and J. A. Washington II. 1991. Antibacterial susceptibility tests: dilution methods, p. 1105-1116. *In*: A. Balows, W. J. Hausler Jr., K. L. Hermann, H. D. Isenberg, and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington D.C.

Savage, D. C. 1982. The effects of stress, diet and environment on the stability of the gastro-intestinal microflora, p. 23-31. *In*: G. E. White (ed.), *Normal and induced changes in the gastro-intestinal microflora in man and animals with special regard to animal performance*. International symposium, Oslo, September 25/26, 1980. Verlag Paul Parey, Berlin and Hamburg.

Sogaard, H. 1973. Incidence of drug resistance and transmissible R factors in strains of *E. coli* isolated from faeces of healthy pigs. *Acta Vet. Scand.* **14**: 381-391.

Tannock, G. W. 1983. Effect of dietary and environmental stress on the gastrointestinal microbiota, p. 517-539. *In*: D. J. Hentges (ed.), *Human intestinal microflora in health and disease*. Academic Press, New York.

Williams, L. P., K. W. Newell. 1970. Salmonella excretion in joy-riding pigs. *Am. J. Public Health.* **60**: 926-929.

Young, B. A. 1981. Cold stress as it affects animal production. *J. Anim. Sci.* **52**: 154-163.

**PAPER II. EFFECTS OF HEAT STRESS ON THE ANTIMICROBIAL DRUG
RESISTANCE OF *ESCHERICHIA COLI* OF THE INTESTINAL FLORA
OF SWINE**

ABSTRACT

The effects of heat stress on the antimicrobial drug resistance of *E. coli* of the intestinal tract of swine were studied in animals from a farm which has not been supplementing antimicrobials in feed for the past 10 years. In one study 10 finisher hogs were heat stressed (34°C) for 24 hours. Antimicrobial resistance prevalence after stress was significantly higher ($P < 0.05$) when compared with pre-stress prevalence for amikacin, ampicillin, cephalothin, neomycin, and tetracycline. This high prevalence of resistance persisted to slaughter which occurred at 10 days post-stress.

In a second study, samples of different sections of the gastrointestinal tract were collected after heat stress and compared with control, non-stressed animals. Results indicated that *E. coli* which colonized the ileum and cecum had a higher prevalence of resistance to ampicillin and tetracycline than the *E. coli* which colonized colon and rectum. When animals were exposed to heat stress, resistance to ampicillin and tetracycline of *E. coli* in the lower digestive tract increased ($P < 0.05$) to a prevalence similar to that observed in the ileum and cecum.

INTRODUCTION

A previous report (Moro et al., 1993) described the effects of cold stress on the antimicrobial resistance of *E. coli* of the intestinal flora of swine. The study demonstrated that after animals were exposed to drastic and sudden drops in temperature (cold stress), an increase in antimicrobial resistance was observed in *E. coli* from their feces against amikacin, ampicillin, cephalothin, neomycin and tetracycline. This increase in prevalence of antimicrobial resistance after exposure to cold stress was nearly consistent over a two year period of sampling.

Gastrointestinal microflora may be disturbed by many forces, including antimicrobial drugs, starvation or other dietary changes, certain changes in environment and possibly by fear and other extreme emotions (Morishita and Ogata, 1969; Holdeman et al., 1973; Moon et al., 1979; Savage, 1982; Tannock, 1983). Changes in antimicrobial resistance after transport and holding stress in swine have been reported (Langlois et al., 1984; Molitoris et al., 1987), as well as after movement of animals into and out of their pens (Hedges et al., 1988). Apparently factors other than feeding or use of antibiotics play a role in establishing or maintaining the antibiotic resistant microflora of animals.

We describe here the effects of heat stress on the antimicrobial resistance of *E. coli* from the intestinal tract of swine from a farm where no antimicrobials have been supplemented in feed for the past ten years.

MATERIALS AND METHODS

Experimental Animals

Finisher hogs (85 kg) from a farm where feed has not been supplemented with antibiotics for over ten years were used in this study. Randomly selected animals from a single pen were transported to the research facility 30 days before the initiation of the study where they were allowed to acclimate in isolated pens at 21°C. Animals had free access to water and were fed the same antimicrobial drug free feed as at farm of origin.

Study 1

Ten finisher hogs were randomly allocated to 3 groups. All 3 groups were exposed to controlled temperatures of 34 °C (heat stress), and 65% relative humidity for 24 hours. Fecal samples were collected on a weekly basis prior to the initiation of treatment as well as every day during and for 10 days after terminating stress. Rectal temperatures were measured before and at end of stress. Animals were then slaughtered at the Iowa State University Meats Laboratory located 3 kilometers from the research facilities. Samples of contents were collected from the colon and rectum after slaughter.

Study 2

Four groups (A, B, C, and D of 3 randomly selected animals each were formed. Groups A, B, and C were exposed to controlled temperatures of 34 °C (heat stress), and 65% relative humidity in the following way: Group A was exposed to heat stress for eight hours and the animals were immediately slaughtered afterwards; group B was exposed for 24 hours and slaughtered immediately afterwards, and group C was also exposed for 24 hours but was slaughtered after one week. Group D was an unexposed control. Rectal temperatures were measured before treatment and again at the termination of stress. Weekly fecal samples were collected prior to the initiation of the study. All groups were slaughtered at the meat laboratory.

Both ends of a 20 to 30 cm segment of ileum, ileocecal valve - cecum, ascending colon, transverse colon, descending colon, and rectum were ligated and the segments removed. Culturing of samples was performed within 2 hours of obtaining the intestinal segments.

Carcass surfaces were cultured at the meats laboratory so that any *E. coli* represented bacteria of fecal origin. The ham, back, shoulder as well as the peritoneal cavity were sampled by swabbing 250 cm² with absorbent cotton swabs and placed in 10ml of PBS.

Microbial Analysis

Ten-fold serial dilutions into sterile buffered saline of approximate 5 g intestinal content material was plated on Tergitol¹ agar with TTC (Triphenyltetrazolium chloride). Plates were incubated at 37 °C for 18-24 hours. Ten smooth *E. coli* colonies were randomly picked from each culture and biochemically identified (Ewing, 1986).

Antimicrobial susceptibility results were determined using a broth dilution breakpoints method in which the antimicrobials were prepared commercially in a microliter plate format² for study 1. For study 2, ampicillin and tetracycline were selected. An agar dilution procedure was utilized with breakpoints at 8 µg/ml for ampicillin and 4 µg/ml for tetracycline³ with each isolate reported as susceptible or resistant (Barry, 1986; Sahm and Washington, 1991; Koneman et al., 1992).

Swabs from carcass samplings were plated on MacConkey agar and incubated for 18-24 hours. *E. coli* isolates were identified biochemically (Ewing, 1986). Comparisons were made between *E. coli* antimicrobial resistance patterns from carcasses and from contents of intestinal segments.

¹ Difco Manual, Difco Laboratories, Detroit, MI.

² SENSITITRE Ltd, The Manor Royal, Crawley, West Sussex, RH10 2PY, England.

³ Sigma Chemical Co., St. Louis, Mo.

Plasmid Profiles

Plasmid DNA was extracted from selected isolates of *E. coli* from different Intestinal segments by a rapid alkaline extraction procedure (Birnboim and Doly, 1979). The plasmid DNA of each isolate was separated by submerged agarose gel electrophoresis, using tris-acetate (40 mM TRIS, 20 mM sodium acetate, 2mM EDTA, pH 7.8) as the buffer system. Plasmid separation was performed by use of 0.75% DNA grade agarose,¹ with electrophoresis performed horizontally at 75 V for 2 hours. The DNA was stained by immersing the gel in 100ml of distilled water containing 10 µl of ethidium bromide (1.0 µl/ml) solution and allowing it to stand at 21°C for 20 minutes. Gels were viewed with a UV transilluminator and were photographed through a red filter.

Statistical Analysis

An analysis of variance procedure was utilized. Differences were considered significant at $P < 0.05$ level (Snedecor and Cochran, 1989).

¹ BRL Life Technologies Inc., Gaithersburg, MD.

RESULTS

Study 1

A significant increase in prevalence of resistance was observed after animals were exposed to heat stress for 24 hours to amikacin, ampicillin, cephalothin, neomycin and tetracycline. Pre and post-stress prevalence of antimicrobial resistance are shown in Table 1. A peak in resistance was obtained immediately following stress. Subsequent samples exhibited a progressive drop in resistance for most of the antimicrobials until final collections were made 10 days after termination of stress (Figure 1).

Table 1. Antimicrobial Resistance Prevalence

Antimicrobial Agent	Pre-Stress	Stress
Amikacin	30%	70%^a
Ampicillin	5%	30%
Cephalothin	35%	80%
Neomycin	2%	40%
Tetracycline	35%	75%

^aBold values were significant ($P < 0.05$).

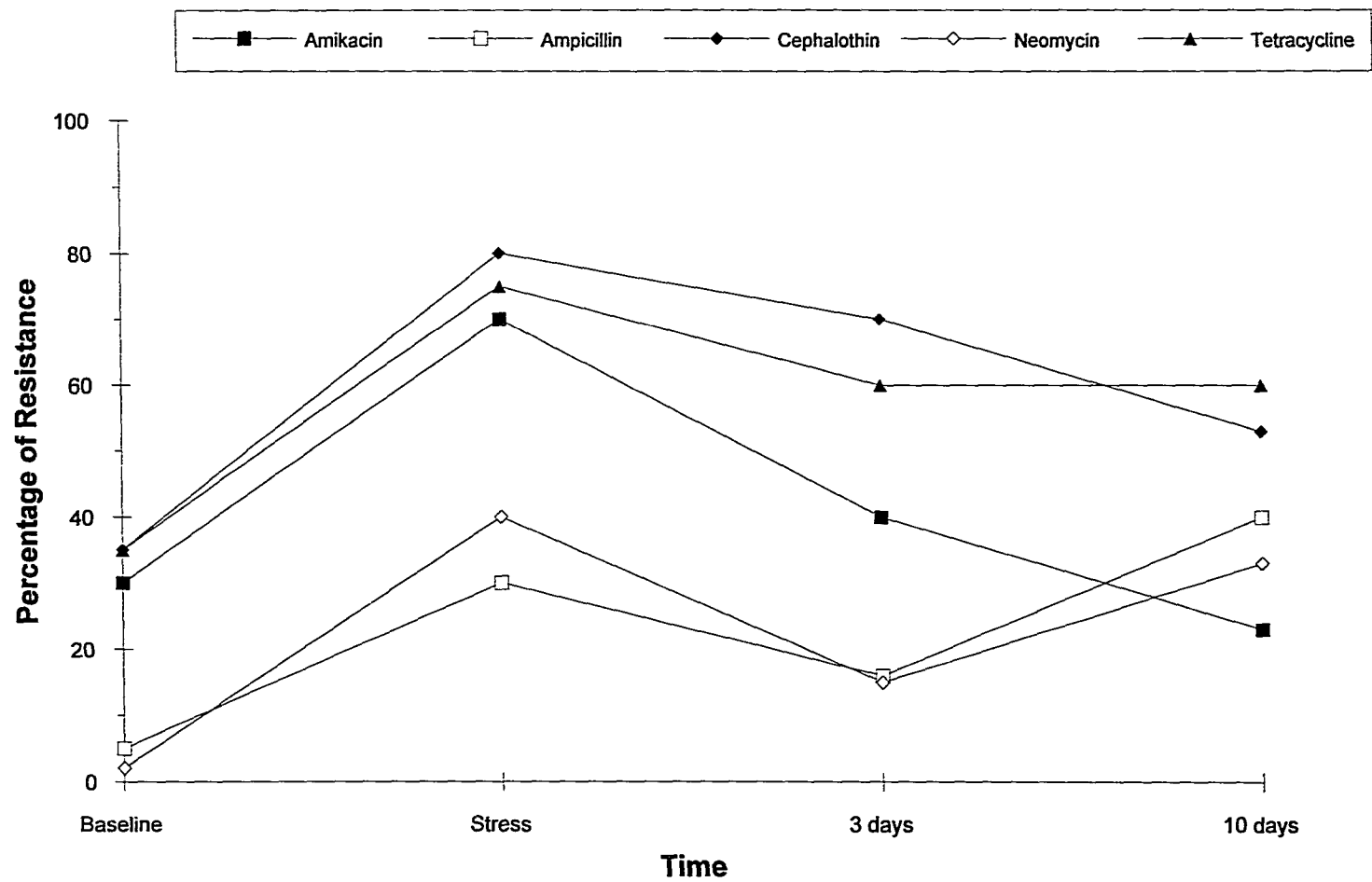


Figure 1. Percentage of Resistant *Echerichia coli* After Heat Stress

Only 25% of the pre-stress isolates showed multiple antimicrobial resistance patterns (Table 2). In contrast multiple resistance was observed in 85% of isolates stressed from swine.

No significant differences between isolates from colon and rectum were found.

Study 2

Percentages of resistance to ampicillin for isolates from the ileum portion of non-stressed (control) swine showed a significantly higher prevalence of resistance (37%) than did isolates from cecum (17%), colon (9%), or rectum (4%). The stressed groups of swine showed significant increases in ampicillin resistance for isolates from the lower intestinal tract (colon and rectum segments) (Figure 2). Non-stressed percentages of resistance to tetracycline were significantly higher for isolates from the upper intestinal tract (ileum and cecum) than from the lower intestinal tract (colon and rectum) as it is shown in Figure 3. All the heat stressed groups (A, B, and C) showed significant increases in tetracycline resistance in isolates from the colon and rectum. Results for isolates from the ileocecal valve were statistically similar to those from the ileum; isolates from the different segments of the colon were all statistically similar.

Antimicrobial resistance patterns for the control group as well as for the stressed group B are shown in Tables 3, 4, 5, and 6. The predominant resistance pattern in isolates from the ileum and cecum in control and stressed animals was to tetracycline, as was true for isolates from the colon and rectum from the control

Table 2. Antimicrobial Resistance Patterns for *E. coli* isolated from Pigs

Resistance Pattern	Pre-stress (100)	Stress (100)
0	20%	5%
AMK	10%	-
CEP	15%	10%
TET	30%	-
AMK + CEP	20%	10%
AMK + TET	-	10%
AMP + TET	5%	-
NEO + TET	-	5%
AMK + NEO + TET	-	5%
AMK + CEP + TET	-	15%
AMK + CEP + NEO + TET	-	10%
AMK + AMP + CEP + TET	-	10%
AMP + CEP + NEO + TET	-	5%
AMK + AMP + CEP + NEO + TET	-	15%

^aNumber of isolates examined.

^bAMK: amikacin; AMP: ampicillin; CEP: cephalothin; NEO: neomycin; TET: tetracycline.

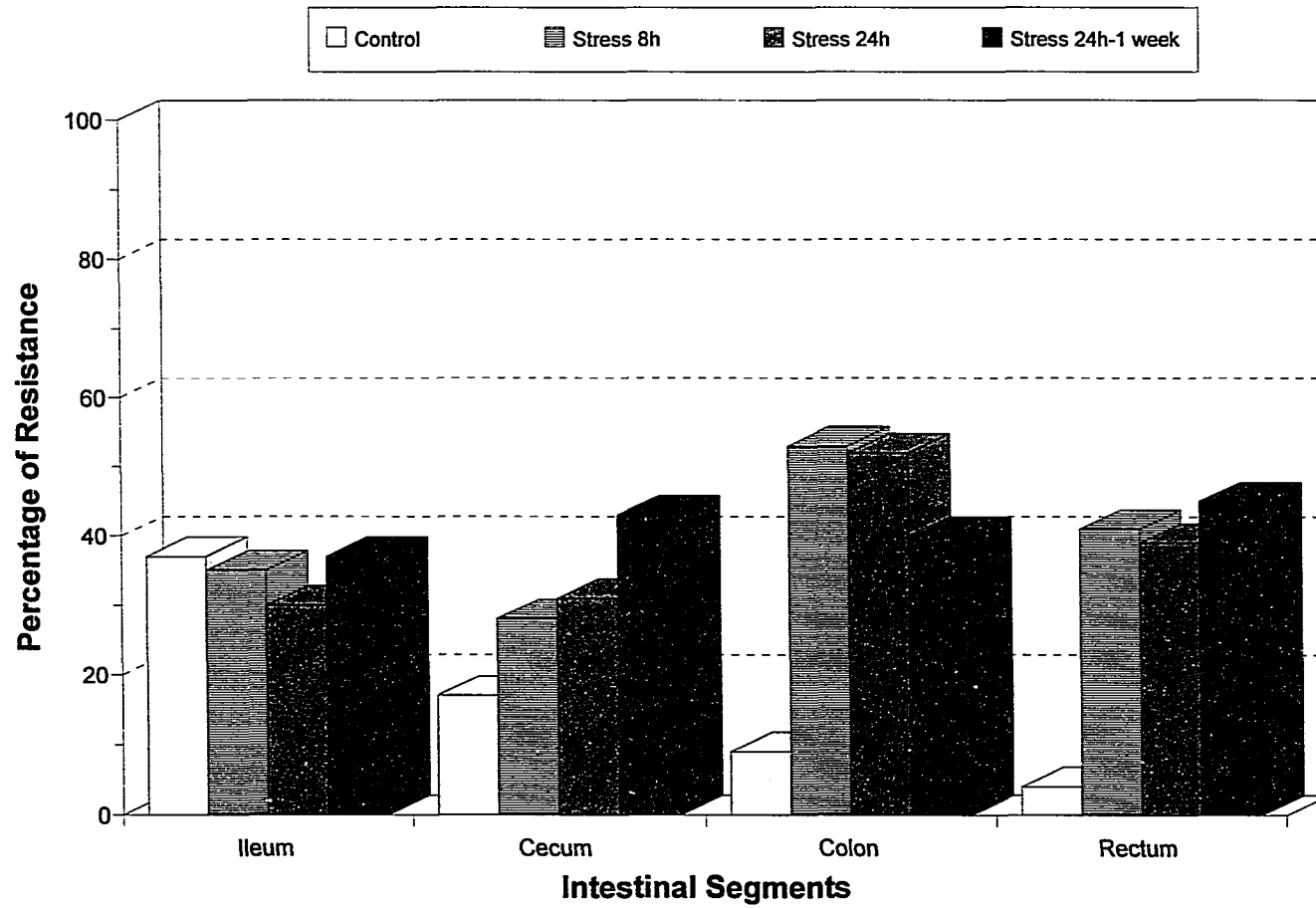


Figure 2. Ampicillin Resistance of *E. coli*. Heat Stress

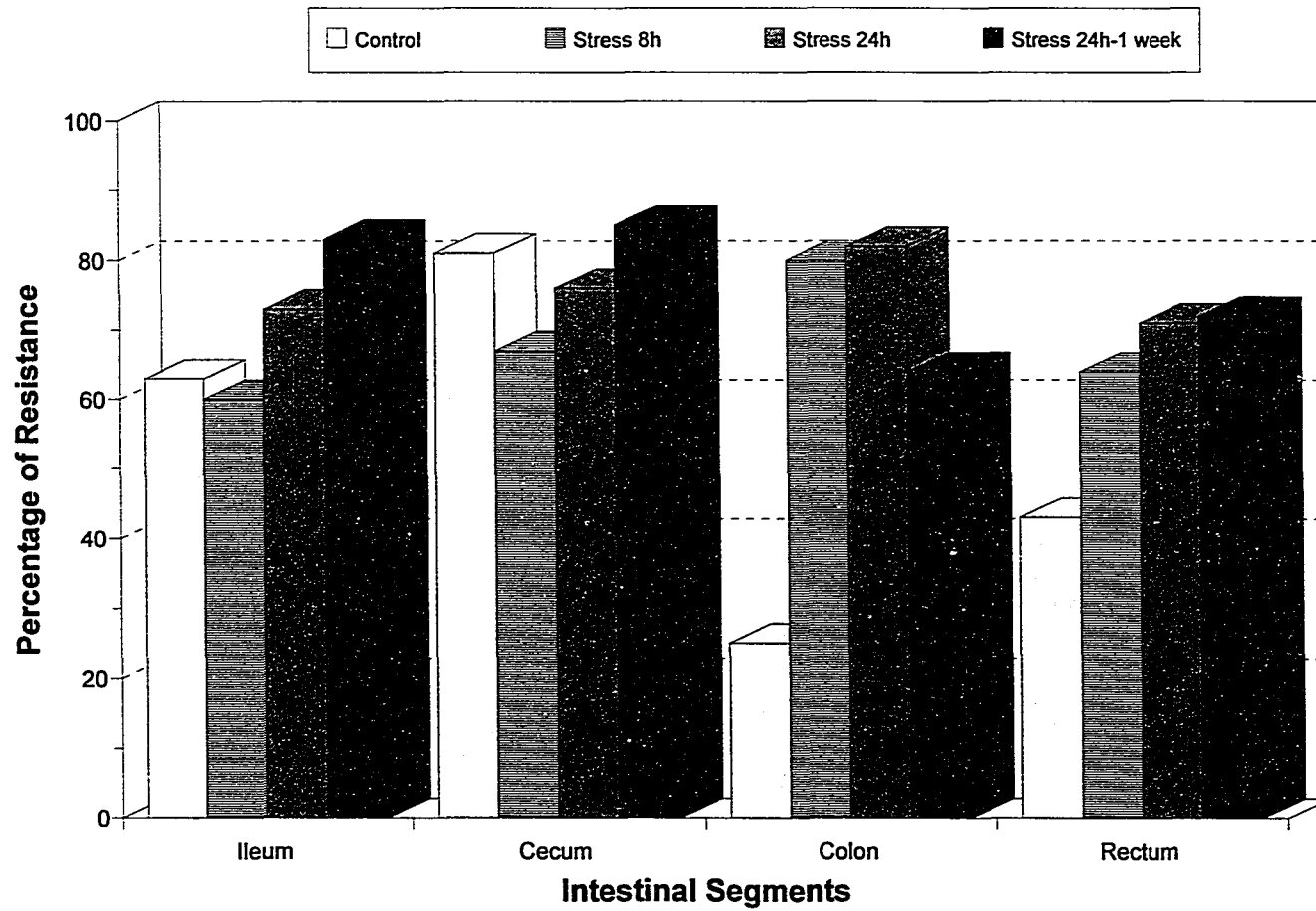


Figure 3. Tetracycline Resistance of *E. coli*. Heat Stress

Table 3. Antimicrobial Resistance Patterns of *E. coli* Isolates from Pig Ileum

Resistance Pattern	Control Group (30) ^a	Stress Group (30)
0	27%	17%
Ampicillin	10%	10%
Tetracycline	37%	53%
Ampicillin + Tetracycline	27%	20%

^aNumber of isolates examined

Table 4. Antimicrobial Resistance Patterns of *E. coli* Isolates from Pig Cecum

Resistance Pattern	Control Group (60)	Stress Group (60)
0	17%	23%
Ampicillin	0%	10%
Tetracycline	63%	43%
Ampicillin + Tetracycline	20%	33%

Table 5. Antimicrobial Resistance Patterns of *E. coli* Isolates from Pig Colon

Resistance Pattern	Control Group (90)	Stress Group (90)
0	73%	16%
Ampicillin	3%	1%
Tetracycline	18%	32%
Ampicillin + Tetracycline	6%	51%

Table 6. Antimicrobial Resistance Patterns of *E. coli* Isolates from Pig Rectum

Resistance Pattern	Control Group (30)	Stress Group (30)
0	50%	29%
Ampicillin	4%	0%
Tetracycline	46%	32%
Ampicillin + Tetracycline	0%	39%

group. Stressed animals, however, had predominantly ampicillin + tetracycline resistance patterns for isolates from the lower intestinal segments.

Results of carcass swabbing and culturing are presented in Figure 8. *E. coli* isolates were obtained from swine in the control group as well as group B (24 h). A significant difference was observed for tetracycline resistance between isolates obtained from the carcasses of the control (40%) versus the stressed group (80%).

E. coli isolates from the colon and rectum of control animals had plasmid profiles that differed from the profiles of isolates from the same segments of stressed animals. In addition, the number of plasmids present per isolate from the colon and rectum of control animals was lower than the number present in isolates from the colon and rectum of stressed animals.

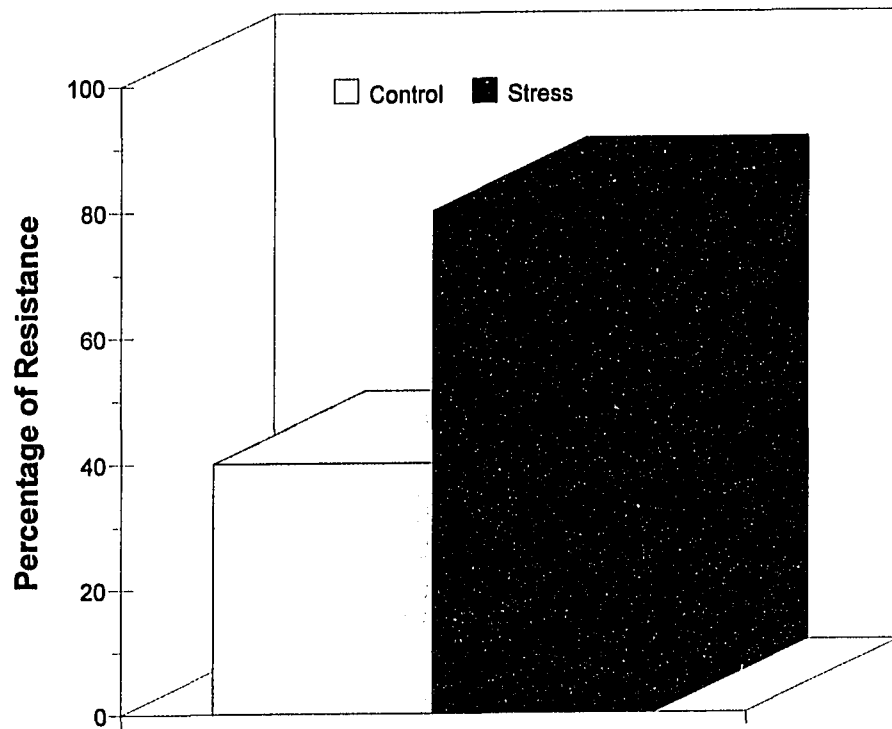


Figure 4. Carcass Sampling. Tetracycline Resistance

DISCUSSION

The heat stress imparted to the animals in these studies was sufficient to induce marked increases in body temperatures and respiratory rates. The zone of effective environmental temperature across which swine can survive is much wider than that in which the thermal environment has no effect on their health, growth and reproduction. Swine are more vulnerable to low temperatures as neonates, but become progressively more susceptible to hot environments as they grow older (Curtis, 1985).

Heat stress in pigs increased prevalence of single and multiple antimicrobial resistance in *E. coli* cultured from the feces of these animals. This high prevalence of resistance tended to persist during the 10 days post-stress at which time the swine in study 1 were slaughtered. Reports of increased levels of prevalence of antimicrobial resistance after the stress of moving and shipping pigs also mention relatively slow return to previous levels of prevalence of antimicrobial resistance (Dwanson et al., 1984; Langlois et al., 1986).

E. coli from the upper intestinal tract (ileum and cecum) had a higher prevalence of ampicillin and tetracycline resistance both in control and stressed animals. After stress, the prevalence of resistance in *E. coli* from the lower intestinal tract (colon and rectum) increased to approach that observed for the upper tract. Further the antimicrobial resistance patterns of *E. coli* isolates from the colon and rectum of stressed animals were very similar to the isolates recovered from the upper

intestinal tract (ileum and cecum) for control and stressed animals. Studies involving young chickens and pigs have indicated that the majority of O serogroups present in feces are not necessarily the same as those colonizing other parts of the intestine (Hinton et al., 1982; 1985). In other words, certain *E. coli* serogroups may colonize specific segments of the gastrointestinal tract. Differences in the proportions of intestinal bacterial populations have been found between populations of the cecum and colon of individual pigs as well as between populations associated with cecal epithelial tissue and populations of the lumen (Allison et al., 1979, Varel et al., 1982). Interestingly sizeable differences between the cecum and colon have been reported for pH and pH in pigs (Allison, 1989).

The complexity of the *E. coli* flora varies between individuals and also within individuals, especially as animals grow older. The population of *E. coli* in the intestinal tract is not static and a turn over of strains has been demonstrated in humans as well as in other animal species (Hinton et al., 1985). Individuals acquire new strains from food, environment, and other animals (Linton and Hinton, 1988).

The explanation for both the instability of the *E. coli* faecal flora and its progressive simplification as animals mature has not yet been sought. Several factors probably operate, including the effect of the genetic constitution of individual *E. coli* strains, the diet, the immune responses of the hosts and the interaction between *E. coli* and other bacterial species comprising the intestinal microflora.

The statistical consideration of sampling *E. coli* isolates from faeces has been considered (Hedges et al., 1977). The more complex the *E. coli* flora the greater the

number of colonies that have to be examined in order to obtain a reasonable estimate of the majority of O serogroups present in the sample; however, small samples have value as indicated in one study which showed that at least 76% of the majority of serotypes could be detected when 10 colonies were selected (Linton et al., 1978).

A higher percentage of tetracycline resistant *E. coli* was obtained when carcasses of stressed animals were swabbed and compared with those of control animals. This indicated that stressed animals were shedding higher numbers of resistant bacteria and they contaminated the carcasses.

Our studies indicate that a population of *E. coli* with a higher prevalence of resistance to antimicrobial drugs such as ampicillin and tetracycline inhabits and colonizes the ileum and cecum. In order for bacteria to colonize the small intestine, they attach to prevent cleaning (removal) by the peristaltic activity. Bacterial populations of the lower ileum, cecum, and rectum form thick layers which are embedded in the mucus gel (Freter, 1988).

Further studies are needed to determine the mechanisms of increase of antimicrobial resistance after heat stress in swine. Nevertheless we hypothesize that when animals are stressed, a series of hormones are secreted that stimulate and significantly increase peristalsis and consequently, an outflow of resistant *E. coli* moves progressively from the ileum and cecum to the colon and rectum.

Environmental stress may increase the prevalence of ampicillin and tetracycline resistant *E. coli* in the lower intestinal tract and consequently augment shedding of these resistant organisms into the environment and food chain.

REFERENCES

- Allison, M. J.** 1989. Characterization of the flora of the large bowel of pigs: a status report. *Anim. Feed Sci. Technol.* **23**: 79-90.
- Allison, M. J., I. M. Robinson, J. A. Bucklin, and G. D. Booth.** 1979. Comparison of bacterial populations of the pig cecum and colon based upon enumeration with specific energy sources. *Appl. Environ. Microbiol.* **37**: 1141-1151.
- Barry, A. L.** 1986. Procedure for testing antimicrobial agents in agar media: theoretical considerations, p. 1-26. *In* V. Lorian (ed.), *Antibiotics in laboratory medicine*, 2nd ed. Williams & Wilkins Co., Baltimore.
- Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* **7**: 1513- 1523.
- Curtis, S. E.** 1985. Physiological responses and adaptations of swine, p. 129-148. *In* M. K. Yousef (ed.), *Stress physiology in livestock. Vol II Ungulates*. CRC Press, Inc., Boca Raton.
- Dawson K. L., B. E. Langlois, T. S. Stahly, and G. L. Cromwell.** 1984. Antibiotic Resistance in anaerobic and coliform bacteria from the intestinal tract of swine fed therapeutic and subtherapeutic concentration of chlortetracycline. *J. Anim. Sci.* **58**: 123-131.
- Ewing, W. H.** 1986. *Edwards and Ewing's Identification of Enterobacteriaceae*. 4th edition. Elsevier Science Publishing Co., Inc., New York.
- Freter, R.** 1988. Mechanisms of bacterial colonization of the mucosal surfaces of the gut, p. 45-60. *In* J. Roth (ed.), *Virulence mechanisms of bacterial pathogens*. American Society for Microbiology, Washington, D.C.
- Hedges, A. J., and A. H. Linton.** 1988. Olaquinox resistance in the coliform flora of pigs and their environment: an ecological study. *J. Appl. Bacteriol.* **64**: 429-443.
- Hedges, A. J., K. Howe, and A. H. Linton.** 1977. Statistical considerations in the sampling of *Escherichia coli* from intestinal sources for serotyping. *J. Appl. Bacteriol.* **43**: 271-280.
- Hinton, M., D. J. Hampson, E. Hampson, and A. H. Linton.** 1985. A comparison of the ecology of *Escherichia coli* in the intestine of healthy unweaned pigs and pigs after weaning. *J. Appl. Bacteriol.* **58**: 471-478.

Hinton, M., V. Allen, and A. H. Linton. 1982. The biotyping of *Escherichia coli* isolated from healthy farm animals. J. Hyg. **88**: 543-555.

Hinton, M., A. H. Linton, and A. J. Hedges. 1985. The ecology of *Escherichia coli* in calves reared as dairy-cow replacements. J. Appl. Bacteriol. **58**: 131-138.

Holdeman, L. V., I. J. Good, and W. E. Moore. 1976. Human fecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress. Appl. Environ. Microbiol. **31**: 359-375.

Koneman, E. W., S. D. Allen, W. M. Janda, P. C. Schreckenberger, and W. C. Winn, Jr. 1992. Antimicrobial susceptibility testing, p. 630-631. *In* E. W. Koneman, S. D. Allen, W. M. Janda, P. C. Schreckenberger, and W. C. Winn, Jr. (ed.), Color atlas and textbook of diagnostic microbiology, 4th ed. J. B. Lippincott Co., Philadelphia.

Langlois, B. E., K. A. Dawson, G. L. Cromwell, and T. S. Stahly. 1986. Antibiotic resistance in pigs following a 13 year ban. J. Anim. Sci. **62**: 18-32 (Suppl. 3).

Linton A. H., B. Handley, and A. D. Osborne. 1978. Fluctuations in *Escherichia coli* O-serotypes in pigs throughout life in the presence and absence of antibiotic treatment. J. Appl. Bacteriol. **44**: 285-298.

Linton, A. H., and M. H. Hinton. 1988. Enterobacteriaceae associated with animals in health and disease. J. Appl. Bacteriol., Symposium Supplement. 71S-85S.

Molitoris, E. D. J. Fagerberg, C. L. Quarles, and M. I. Krlchevsky. 1987. Changes in antimicrobial resistance in fecal bacteria associated with pig transit and holding times at slaughter plants. Appl. Environ. Microbiol. **53**: 1307-1310.

Moon, H. W., P. Y. Fung, R. E. Isaacson, and G. D. Booth. 1979. Effects of age, ambient temperature, and heat-stable *Escherichia coli* enterotoxin on intestinal transit in infant mice. Infect. Immun. **25**: 127-132.

Morishita, Y., and M. Ogata. 1970. Studies on the alimentary flora of pigs. V. Influence of starvation on the microbial flora. Jap. J. Vet. Sci. **32**: 19-24.

Moro, M. H., G. W. Beran, L. J. Hoffman, and R. W. Griffith. 1993. Effects of cold stress on the antimicrobial drug resistance of *Escherichia coli* of the intestinal flora of swine. (To be submitted) [Paper I of this dissertation].

Sahn, D. F., and J. A. Washington II. 1991. Antibacterial susceptibility tests: dilution methods, p. 1019-1021. *In*: A. Balows, W. J. Hausler Jr., K. L. Hermann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington D.C.

Savage, D. C. 1982. The effects of stress, diet and environment on the stability of the gastro-intestinal microflora, p. 23-31. *In*: G. E. White (ed.), Normal and induced changes in the gastro-intestinal microflora in man and animals with special regard to animal performance. International symposium, Oslo, September 25/26, 1980. Verlag Paul Parey, Berlin and Hamburg.

Snedecor, G. W., and W. G. Cochran. 1989. Statistical methods, 8th ed. Iowa State University Press, Ames.

Tannock, G. W. 1983. Effect of dietary and environmental stress on the gastrointestinal microbiota, p. 517-539. *In*: D. J. Hentges (ed.), Human intestinal microflora in health and disease. Academic Press, New York.

Varel, V. H., W. G. Pond, J. C. Pekas, and J. T. Yen. 1982. Influence of high-fiber diet on bacterial populations in intestinal tracts of obese and lean genotype pigs. *Appl. Environ. Microbiol.* **44**: 107-112.

**PAPER III. EFFECTS OF CHANGES IN TRANSIT TIME AND HEAT STRESS
ON INTESTINAL *ESCHERICHIA COLI* OF SWINE**

ABSTRACT

An investigation was made (1) to determine the effects of reducing intestinal transit time by injecting a drug that increases intestinal motility on the antimicrobial resistance of *E. coli* of the intestinal flora of swine, and (2) to determine the effects of heat stress on the intestinal transit time in swine. For each study two groups of 3 randomly selected finisher hogs each were formed (treated and control groups). In the first study, induction of increased motility and peristalsis was obtained using an intramuscular injection of the cholinergic drug Neostigmine methylsulfate. *E. coli* isolates were obtained from the ileum, cecum, colon and rectum after animals were slaughtered. A higher prevalence of ampicillin resistant *E. coli* was found in the cecum (35%) than in other segments of the intestinal tract. In treated animals, prevalence of resistance increased for organisms from colon and rectum. For tetracycline resistance, similar results were obtained.

A second study was devised to determine intestinal transit time using Chromium-EDTA as marker eight hours after administration of the marker to control and heat stressed animals, swine were killed and samples were collected throughout the intestinal tract (duodenum to rectum). Results indicated a reduced transit time for the stressed group. Our findings corroborate the initial hypothesis that an outflow of resistant organisms moves from the upper tract (ileum and cecum) to the lower tract (colon and rectum).

INTRODUCTION

There have been some reports describing increasing antimicrobial drug resistance of *E. coli* of the intestinal flora of swine after animals were exposed to some type of stress i. e. shipping and/or moving (Dawson et al., 1984; Langlois et al., 1986; Molitoris et al., 1987). No scientific explanation has been found for this phenomenon. In a previous paper (Moro et al., 1993) an increased prevalence in antimicrobial drug resistance in enteric *E. coli* was observed when animals were exposed to cold or hot environments. Furthermore, significant differences were found in resistant populations of *E. coli* in the ileum and cecum versus those of the colon and rectum of control animals. Percentages of antimicrobial resistant *E. coli* isolates from the colon and rectum had values similar to those observed in the upper intestinal tract after pigs were exposed to heat stress.

There is increasing evidence that different stressors affect gastrointestinal motility in humans (Stanghellini et al., 1983; Valori et al., 1986) and animals (Garrick et al., 1986; Williams et al., 1987; 1988; Lenz et al., 1988). Stress stimulates colonic motor activity in humans (Narducci et al., 1985). Restraint of rats at room temperature or in cold environments stimulated colonic transit time and fecal pellet output (Williams et al., 1987; Lenz et al., 1988; Barone et al., 1990). Environmental and dietary stress have also been shown to increase the number of coliforms in the jejunum, ileum, and cecum in mice and decrease lactobacilli (Tannock and Savage, 1974). Increases in the number of coliforms inhabiting the small intestine have been reported in pigs exposed

to heat or cold stress (Sinkovics, 1975; Kovacs et al., 1976).

The present investigation was developed to determine whether (1) changing transit time by administration of drugs which affect intestinal motility would duplicate the previous observation of increased antimicrobial resistance of *E. coli* from the lower intestinal flora of swine, and (2) intestinal transit time would be affected when animals were exposed to heat stress.

MATERIALS AND METHODS

Experimental Animals

Finisher pigs (85 kg) from a farm where no antibiotics had been added to feed for ten years were used in this study. Randomly selected animals from the same pen were transported to the research facility 30 days prior to the initiation of the study and maintained in isolated pens at 21°C. Animals had free access to water and were fed the same feed as at the farm of origin. Two different studies were performed.

Study 1

Six pigs were randomly allocated in 2 groups (3 animals per group). Fecal samples were collected on a weekly basis prior to the initiation of the study for determination of baseline prevalence of ampicillin and tetracycline resistance of intestinal *E. coli*. Intestinal hypermotility was induced by the use of Neostigmine methylsulfate¹ (0.03mg/kg). Two intramuscular injections were administered 17 and 9 hours prior to slaughter of the experimental group. Control animals were injected with distilled water at the same time intervals. Animals were slaughtered at the Iowa State University Meats Laboratory. Samples of 20 to 30 cm segments of ileum, cecum, colon, and rectum were ligated at both ends and removed.

Ten-fold serial dilutions using 5 g of intestinal contents were prepared in sterile

¹ Stiglyn (Neostigmine Methylsulfate Injection), Pitman-Moore, Inc. Mundelein, IL.

buffered saline and cultured on duplicate plates of Tergitol¹ agar with TTC (triphenyltetrazolium chloride). Plates were incubated at 37 °C for 18-24 hours. Twenty *E. coli* colonies were randomly picked from each plate and biochemically identified (Ewing, 1986).

Antimicrobial susceptibility testing was performed with an agar dilution technique (Barry, 1986; Sahm and Washington, 1991; Koneman et al., 1992). Breakpoints for ampicillin were 8 µg/ml and 4 µg/ml for tetracycline² with each isolate reported as susceptible or resistant.

Counts for that portion of the total coliform population (lactose positive enteric bacteria) resistant to ampicillin and tetracycline were determined by plating in duplicate ten-fold dilutions of intestinal contents as described above on MacConkey agar and MacConkey agar plus 25 µg ampicillin/ml and 25 µg tetracycline/ml. Plates were incubated at 37 °C for 24 hours and lactose positive colonies were counted. The percentage of the coliform population resistant to ampicillin and tetracycline was determined by dividing the count obtained on MacConkey agar plus 25 µg ampicillin/ml and 25 µg tetracycline/ml by the count obtained on MacConkey agar without antibiotics and multiplying by 100 (Langlois et al., 1978).

¹ Difco manual, Difco Laboratories, Detroit, MI.

² Sigma Chemical Co., St. Louis, MO.

Study 2

Six pigs were randomly allocated in 2 groups (3 animals per group). Fecal samples were collected on a weekly basis prior to the initiation of the study for determination of baseline levels of ampicillin and tetracycline resistance. The stressed group was exposed to controlled temperatures of 34°C (heat stress), and 65% relative humidity for 15 hours. Rectal temperatures were registered before and during heat stress. For determination of intestinal transit time a soluble marker, CrEDTA¹, was used. Fifty g of CrCl₃6H₂O and 62 g disodium EDTA were boiled in 500 ml distilled water for 1 hour. A small excess of EDTA was neutralized with 10 ml of 1 M calcium chloride and the pH was brought to 7.0 (Binnerts et al., 1968; Gregory et al., 1985). A 45 ml dose of this CrEDTA was given by stomach tube to each control and stressed animal 8 hours before slaughter. Immediately following slaughter at the university facilities, ligated samples of 20 to 25 cm of duodenum, jejunum, ileum, cecum, centripetal section of the ascending colon (CA), centrifugal section of the ascending colon (CB), transverse colon (CC), descending colon (DC), and rectum were collected, placed in plastic bags and placed in refrigeration. For chromium determination, intestinal contents were removed from each segment by alternating compressing and stretching the segments to expel all removable content which were then transferred to vessels large enough to hold the contents. The mucosal linings of the segments were then washed with distilled water to assure complete removal of

¹ Chromium chloride and Ethylenediaminetetraacetic acid, Sigma Chemical Co. St. Louis, MO.

matter. The washings were added to the contents in the beakers or dishes. Samples were dried on a hot plate and subsequently ashed. The specimens were removed from the containers using concentrated hydrochloric (HCL) acid and finally washed and diluted with 50 ml each of 2N hydrochloric acid (Stahr, 1991). The extracts were then analyzed by atomic absorption, diluting them as required. The amount of chromium found was reported as micrograms per segment.

Statistical Analysis

Mean coliform counts were log transformed before statistical analysis. The data were analyzed by ANOVA. Differences were considered significant at $P < 0.05$ level (Snedecor and Cochran, 1989).

RESULTS

Study 1

A significant increase of the percentage of ampicillin and tetracycline resistant *E. coli* was observed after animals were injected with neostigmine for isolates of the colon (55% for control group versus 70% for treated group) and rectum (58% and 64%) respectively. Figure 1 shows ampicillin resistance for the control and treated groups. Within control animals, 18% and 23% respectively of isolates from the colon and rectum had ampicillin resistance. The treated group showed no significant differences when compared with the control group for isolates from ileum and cecum but isolates from the colon and rectum had ampicillin resistance prevalence significantly higher for the treated group. Tetracycline resistance prevalence for the ileum and cecum was significantly higher than for the colon and rectum isolates of control animals. Treated animals did have similar prevalence of tetracycline resistance for ileum and cecum (68% and 78%). *E. coli* isolates from the colon and rectum had significantly higher prevalence of tetracycline resistance for the treated animals than the control group (Figure 2).

Prevalence of ampicillin and tetracycline resistance in total coliforms was similar to that obtained for *E. coli*.

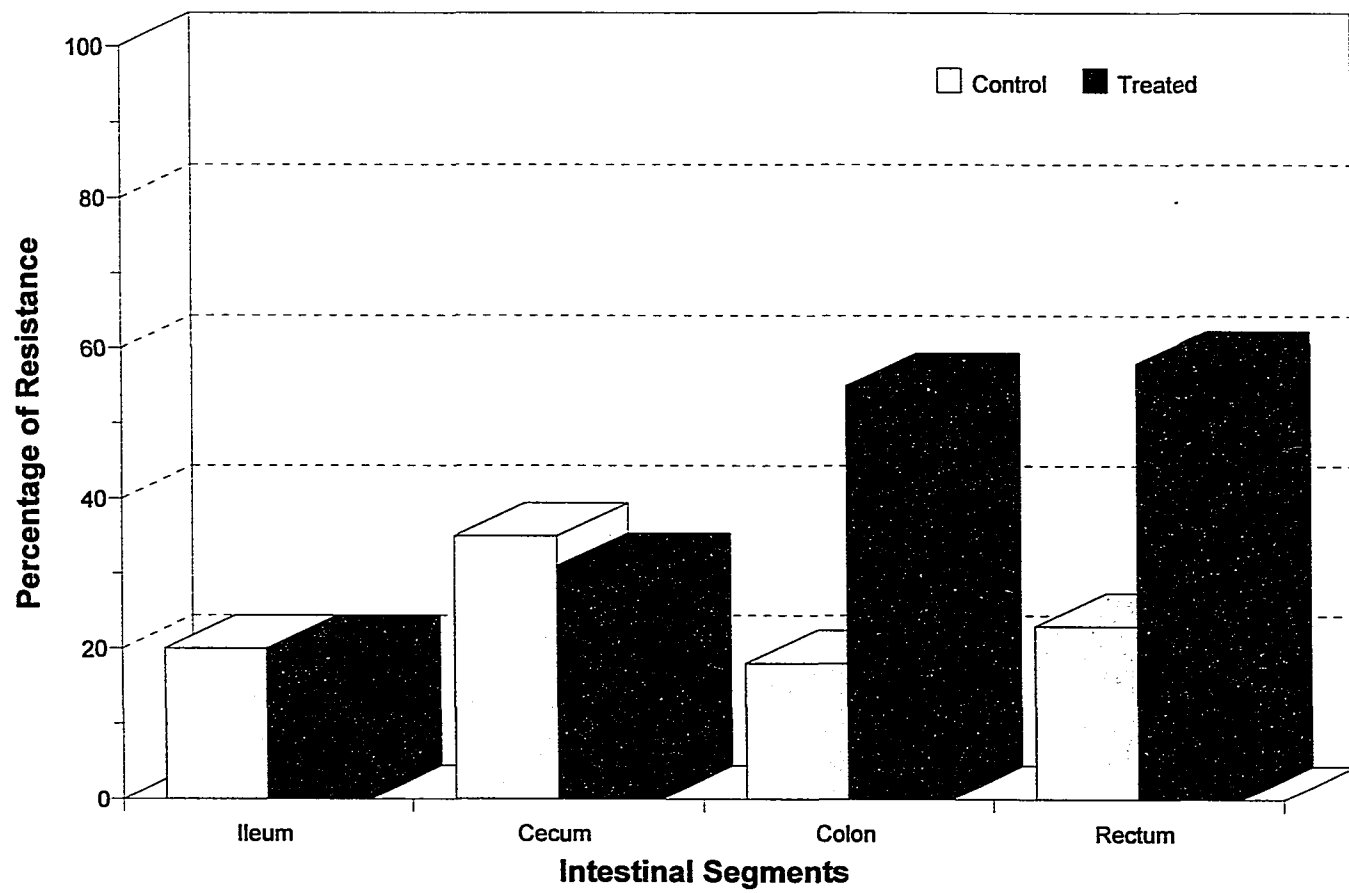


Figure 1. Motility Induction. Ampicillin Resistance of *E. coli*.

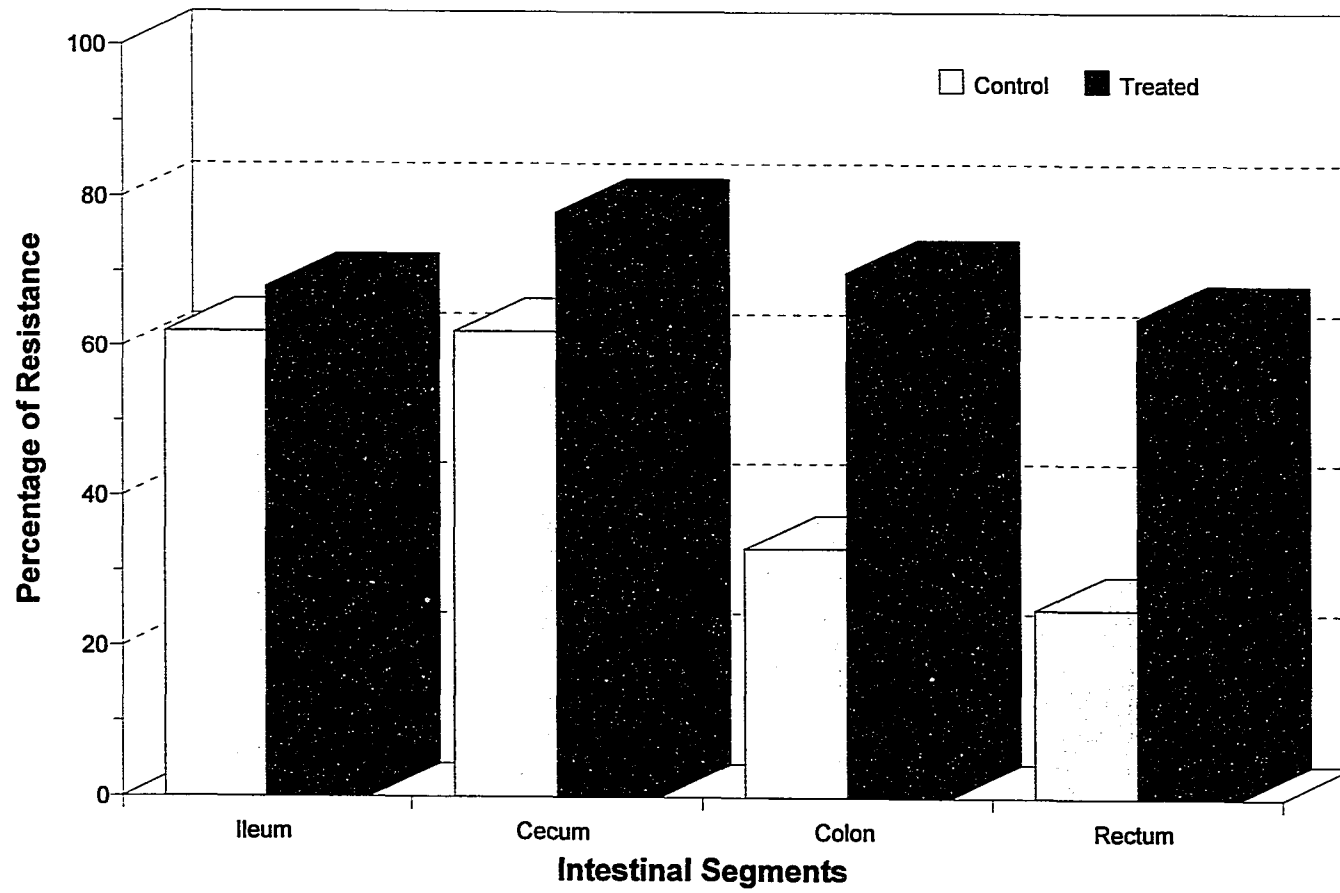


Figure 2. Motility Induction. Tetracycline Resistance of *Echerichia coli*.

Study 2

Most of the chromium recovered in the control group was located in the cecum (60%). Colon A had 19% and colon B had 16% of the total. Other sampled segments had values between 0.5 to 2%. Forty-two percent of the recovered chromium from the stressed group was located in the cecum, 10% from colon A, 34% from colon B, and 8% from colon C. All these values were significant when compared with the control group. Percentages for the other segments ranged from 0.5% to 3%. Figure 3 shows both groups of animals and percentages of total chromium recovered.

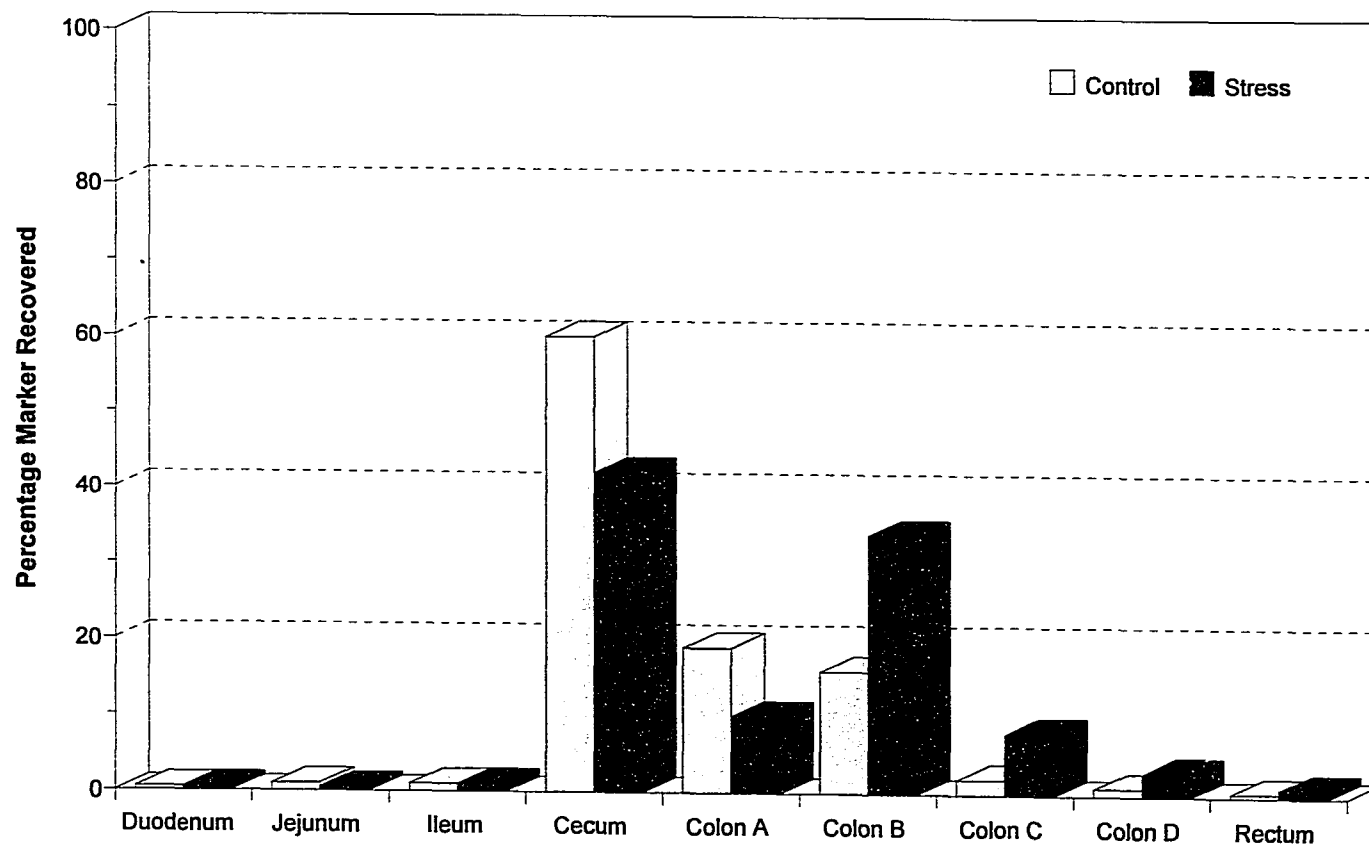


Figure 3. Transit Time and Heat Stress. Percentage of Chromium Recovered from the Intestinal Tract

DISCUSSION

In the first part of the study we reduced transit time by use of Neostigmine, an anticholinesterase agent that causes contraction of the smooth muscle resulting in an increase in the gastrointestinal motility and peristaltic movements of the intestinal tract. The frequency and strength of peristalsis increases and a shorter transit time occurs. Evidence of its action was noted in two of the treated animals which had a higher water content in their feces. Neostigmine is not known to influence microbial activity directly.

Prevalence of ampicillin and tetracycline resistance of *E. coli* for all the segments studied was very similar to that observed in a previous study (Moro et al., 1993), where animals were exposed to heat stress. Control as well as treated animals showed a higher prevalence of ampicillin and tetracycline resistance in isolates from the ileum and cecum. Isolates from the colon and rectum of treated animals had percentages of resistance similar to that found in the ileum and cecum. Changes in the intestinal microbial flora mass have been reported in humans when transit time was reduced by administration of drugs (Stephen et al., 1987). We did not detect a significant difference in coliform counts between control and treated groups of pigs. In the habitat of the lumen of the small intestine, the rate of bacterial removal exceeds the maximum rate of multiplication of probably all known bacteria (Freter, 1983). Therefore this habitat can be colonized only by bacteria that are capable of adhering to the gut wall. In the large intestine the rate of removal is considerably slower and

colonization is possible without recourse to adhesion to the gut wall. We believe that an increase in intestinal motility and peristalsis produces an outflow of resistant *E. coli* organisms from the upper to the lower segments of the intestinal tract.

Evidence of decreased transit time when animals are under heat stress was demonstrated in the second part of the study. Physical restraint in the rat has been shown to produce neuroendocrine responses like increases in plasma concentration of adrenocorticotrophic hormones, β endorphins, cortisol, epinephrine, norepinephrine and glucose (Williams et al., 1988; Lenz et al., 1989), as well as inhibition of small bowel transit and stimulation of large bowel transit (Williams et al., 1987; 1988). In a study of long duration stress in rats a normal motility pattern was restored in the small intestinal tract but alterations were still present in the colon long after the stress stimulus (Wittmann et al., 1990).

Corticotropin Releasing Factor (CRF) is released during stress (Plotsky and Vale, 1984) and has been shown to be involved in the alteration of gastric motility induced by physical and psychological stress in animals (Gue et al., 1991; Monnikes et al., 1993). Furthermore when injected into the brain, CRF markedly accelerates large bowel transit. CRF has been proposed as a central nervous system mediator of these effects (Williams et al., 1987). There is evidence that CRF may influence gastrointestinal motility through the modulation of the parasympathetic nervous system; therefore, an increase in central cholinergic activity is known to activate colonic motility (Boom et al., 1965).

In conclusion we have demonstrated that heat stress in swine increased

propulsion of intestinal content causing a reduction in transit time. We believe that the upper gastrointestinal tract may act as a reservoir for resistant *E. coli* organisms even after many years of no direct supplementation of antimicrobials in feed. When animals are stressed, resistant bacteria from the upper tract flow to the lower tract and are shed into the environment.

REFERENCES

- Barone, F. C., J. F. Deegan, W. J. Price, P. J. Fowler, J. D. Fondacaro, and H. S. Ormsbee III.** 1990. Cold-restraint increases rat fecal pellet output and colonic transit. *Am. J. Physiol.* **258**: G329-G337.
- Barry, A. L.** 1986. Procedure for testing antimicrobial agents in agar media: theoretical considerations, p. 1-26. *In* V. Lorian (ed.), *Antibiotics in laboratory medicine*, 2nd ed. Williams & Wilkins Co., Baltimore.
- Binnerts, W. T., A. Klooster, and A. M. Frens.** 1968. Soluble chromium indicator measured by atomic absorption in digestion experiments. *Vet. Rec.* **82**: 470. (Abstract.)
- Boom, R., G. Chavez-Ibarra, J. J. Del Villar, and R. Hernandez-Pron.** 1965. Changes of colonic motility induced by electrical and chemical stimulation of the forebrain and hypothalamus in cats. *Int. J. Neuropharmacol.* **4**: 169-175.
- Dawson K. L., B. E. Langlois, T. S. Stahly, and G. L. Cromwell.** 1984. Antibiotic Resistance in anaerobic and coliform bacteria from the intestinal tract of swine fed therapeutic and subtherapeutic concentration of chortetracycline. *J. Anim. Sci.* **58**: 123-131.
- Ewing, W. H.** 1986. *Edwards and Ewing's Identification of Enterobacteriaceae*. 4th edition. Elsevier Science Publishing Co., Inc., New York.
- Freter, R.** 1983. Mechanisms that control the microflora in the large intestine, p. 33-54. *In* D. J. Hentges (ed.), *Human intestinal microflora in health and disease*. Academic Press, New York.
- Garrick, T., F. W. Leung, S. Buack, K. Hirabayashik, and P. H. Guth.** 1986. Gastric motility is stimulated but overall flow is unaffected during cold restraint in the rat. *Gastroenterology* **91**: 141-148.
- Gregory, P. C., S. J. Miller, and A. C. Brewer.** 1985. The relation between food intake and abomasal emptying and small intestinal transit time in sheep. *Br. J. Nutr.* **53**: 373-380.
- Gue, M., J. L. Junien, and L. Bueno.** 1991. Conditioned emotional response in rats enhances colonic motility through the central release of corticotropin-releasing factor. *Gastroenterology* **100**: 964-970.

Koneman, E. W., S. D. Allen, W. M. Janda, P. C. Schreckenberger, and W. C. Winn, Jr. 1992. Antimicrobial susceptibility testing, p. 630-631. *In* E. W. Koneman, S. D. Allen, W. M. Janda, P. C. Schreckenberger, and W. C. Winn, Jr. (ed.), Color atlas and textbook of diagnostic microbiology, 4th ed. J. B. Lippincott Co., Philadelphia.

Langlois, B. E., K. A. Dawson, G. L. Cromwell, and T. S. Stahly. 1986. Antibiotic resistance in pigs following a 13 year ban. *J. Anim. Sci.* **62**: 18-32 (Suppl. 3).

Langlois, B. E., G. L. Cromwell, and V. W. Hays. 1978. Influence of chlortetracycline in swine feed on reproductive performance and on incidence and persistence of antibiotic resistant enteric bacteria. *J. Anim. Sci.* **46**: 1369-1382.

Lenz, H. J. 1989. Neurohormonal pathways mediating stress-induced changes in rat gastrointestinal transit. *Gastroenterology* **97**: 216-218.

Lenz H. J., A. Raedler, H. Greten, W. W. Vale, and J. E. Rivier. 1988. Stress-induced gastrointestinal secretory and motor responses in rats are mediated by endogenous corticotropin-releasing factor. *Gastroenterology* **95**: 1510-1517.

Molitoris, E. D. J. Fagerberg, C. L. Quarles, and M. I. Krichevsky. 1987. Changes in antimicrobial resistance in fecal bacteria associated with pig transit and holding times at slaughter plants. *Appl. Environ. Microbiol.* **53**: 1307-1310.

Monnikes, H., G. Schmidt, and Y. Tache. 1993. Psychological stress-induced accelerated colonic transit in rats involves hypothalamic corticotropin-releasing factor. *Gastroenterology* **104**: 716-723.

Moro, M. H., G. W. Beran, L. J. Hoffman, and R. W. Griffith. 1993. Effects of cold stress on the antimicrobial drug resistance of *Escherichia coli* of the intestinal flora of swine. (to be submitted) [Paper I of this dissertation].

Moro, M. H., G. W. Beran, R. W. Griffith, and L. J. Hoffman. 1993. Effects of heat stress on the antimicrobial drug resistance of *Escherichia coli* of the intestinal flora of swine. (to be submitted) [Paper II of this dissertation].

Narducci, F., W. J. Snape, W. M. Battle, R. L. London, and S. Cohen. 1985. Increased colonic motility during exposure to a stressful situation. *Dig. Dis. Sci.* **30**: 40-44.

Kovacs, F., G. Sinkovics, and P. Rafal. 1976. Effects of some environmental factors on the intestinal flora of piglets. *Magyar Allatorv. Lapja* **1**: 8-12.

Plotsky, P. M., and W. Vale. 1984. Hemorrhage-induced secretion of corticotropin-releasing factor-like immunoreactivity into the rat hypophyseal portal circulation and its inhibition by glucocorticoids. *Endocrinology* **114**: 164-169.

Sahn, D. F., and J. A. Washington II. 1991. Antibacterial susceptibility tests: dilution methods, p. 1019-1021. *In*: A. Balows, W. J. Hausler Jr., K. L. Hermann, H. D. Isenberg, and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington D.C.

Sinkovics, G. 1975. Effect of certain environmental factors on the gut flora of pigs, p. 338-342. *In* F. Kovacs and P. Rafai (ed.), *Progress in animal hygiene*. Akademiai Kiado, Budapest.

Snedecor, G. W., and W. G. Cochran. 1989. *Statistical methods*, 8th ed. Iowa State University Press, Ames.

Stahr, H. M. 1991. *Analytical methods in toxicology*. John Wiley and Sons, New York.

Stanghellini, V., J. R. Malagelada, A. R. Zinsmeister, V. L. Go, and P. C. Kao. 1983. Stress-induced gastroduodenal motor disturbances in humans: possible humoral mechanisms. *Gastroenterology* **85**: 83-91.

Stephen, A. M., H. S. Wiggins, and J. H. Cummings. 1987. Effect of changing transit time on colonic microbial metabolism in man. *Gut* **28**: 60-609.

Tannock, G. W., and D. C. Savage. 1974. Influences of dietary and environmental stress on microbial populations in the murine gastrointestinal tract. *Infect. Immun.* **9**: 591-598.

Valori, R. M., D. Kumar, and D. L. Wingate. 1986. Effects of different types of stress and of prokinetic drugs on the control of the fasting motor complex in humans. *Gastroenterology* **90**: 890-900.

Williams, C. L., R. G. Villar, J. M. Peterson, and T. F. Burks. 1988. Stress-induced changes in intestinal transit in the rat: a model for irritable bowel syndrome. *Gastroenterology* **94**: 611-621.

Williams, C. L., J. M. Peterson, R. G. Villar, and T. F. Burks. 1987. Corticotropin releasing factor directly mediates colonic responses to stress. *Am. J. Physiol.* **253**: G582-586.

Wittmann, T., F. Crenner, F. Angel, L. Hanusz, C. Ringwald, and J. F. Grenier.
1990. Long duration stress: Immediate and late effects on small and large bowel motility in rat. *Dig. Dis. Sci.* **35**: 495-500.

GENERAL SUMMARY AND DISCUSSION

In the first section of this dissertation, the antimicrobial drug resistance of intestinal *E. coli* of swine from a farm where no antimicrobials are supplemented in feed was determined. Initially, antimicrobial drug resistance determinations were made from pigs of different age groups (growers, finishers, gilts, and sows). Afterwards only finishers were sampled over a period of 2 years. Samples were collected over periods considered seasonally normal and stable (baseline) as well as during drastic drops in environmental temperature (cold stress). Baseline resistance levels tended to be higher for younger animals (growers) than for animals older than 4 months of age (finishers, gilts, and sows). The relationship of age and antimicrobial resistance of *E. coli* from the intestinal tract is difficult to explain since young pigs have been exposed to the microflora of their dams. Other investigators, however, (Guinee, 1972; Linton et al., 1972; Sogaard, 1973; Linton et al., 1975; Hinton et al., 1985; 1986) have reported higher levels of resistance and greater incidence of multiple resistance in young animals and children. Younger animals may be colonized more readily than the older animals by antimicrobial drug resistant organisms in the absence of any antimicrobial selection pressure (Hartley and Richmond, 1975; Petrocheilou et al., 1976; 1977). This suggests that the survival of these strains is dependent on other characteristics of the strains more than on the resistance genes (Hinton, 1986).

Measurement and interpretation of natural environmental stress on animals may be a difficult task due to the occurrence of different factors that may confound results;

however, we observed significant increases in the percentage of ampicillin and tetracycline resistant enteric *E. coli* almost all the times animals were under cold stress.

The second section was designed to further determine and evaluate the findings observed when swine were subjected to naturally occurring cold stress in a more controlled situation. Heat stress was used as an environmental stressor due to the susceptibility of pigs to high temperatures. In the first part of this study 10 finisher hogs were heat stressed (34 °C) for 24 hours. Antimicrobial resistance prevalence after stress was significantly elevated in the enteric *E. coli* when compared with pre-stress prevalence against amikacin, ampicillin, cephalothin, neomycin, and tetracycline. This high prevalence of resistance persisted up to slaughter of the animals 10 days post-stress. In the second part of this study samples of different sections of the gastrointestinal tract were collected and cultured after the experimental heat stress and compared with samples cultured from control animals. *E. coli* isolates from the upper intestinal tract (ileum and cecum) had a higher prevalence of ampicillin and tetracycline resistance both in control and stressed animals. After stress, the prevalence of resistance in *E. coli* from the lower intestinal tract (colon and rectum) increased to a prevalence similar to that observed in organisms from the upper tract.

Studies involving young chickens and pigs had indicated that the majority of *E. coli* O serogroups organisms present in feces do not necessarily represent those strains colonizing other parts of the intestine (Hinton et al., 1982; 1985). Differences in the proportions of intestinal bacteria species have been reported between

populations inhabiting the cecum and colon of individual pigs, as well as between populations associated with cecal epithelial tissue and in the lumen of the cecum (Allison et al., 1979; Varel et al., 1982).

A high prevalence of tetracycline resistance in *E. coli* was observed when carcasses of stressed animals were swabbed and *E. coli* isolates were compared with cultures on carcasses of control animals. This would indicate that stressed animals were shedding a higher number of resistant bacteria and these were contaminating the carcasses.

Our studies indicated that a population of *E. coli* with a high prevalence of resistance to antimicrobial drugs such as ampicillin and tetracycline inhabits and colonizes the ileum and cecum even in swine not exposed to these drugs. At this point we hypothesized that a probable mechanism of increase prevalence of antimicrobial resistance in the lower digestive tract and feces of stressed swine was an outflow of resistant *E. coli* organisms from the upper tract. When animals are under stress, a progressive and accelerated movement of luminal contents from the ileum and cecum to the colon and rectum occurs.

The third section was designed to test the outflow hypothesis. In the first part of the study we reduced intestinal transit time by use of neostigmine, an anticholinesterase agent that causes contraction of smooth muscle which causes an increase in the gastrointestinal motility and peristaltic movements of the intestinal tract. Ampicillin and tetracycline resistance in *E. coli* for all the intestinal segments studied in the neostigmine-treated swine was very similar to that observed when animals were

exposed to heat stress (PAPER II). Control as well as treated animals showed a higher prevalence of ampicillin and tetracycline resistance for isolates from the ileum and cecum. Isolates from the colon and rectum of treated animals had percentages of resistance similar to that found in the ileum and cecum. Physical as well as psychological stress has been demonstrated to affect gastrointestinal motility in humans (Stanghellini et al., 1983; Valori et al., 1986) and animals (Garrick et al., 1986; Williams et al., 1987; 1988; Lenz et al., 1988), and changes in the intestinal microflora mass have been reported in humans when intestinal transit time was reduced by administration of drugs (Stephen et al., 1987). We did not detect a significant difference in coliform counts between control and treated pigs. Based on our findings we believe that an increase in intestinal motility and peristalsis produced an outflow of resistant *E. coli* organisms from the upper to the lower segments of the intestinal tract.

Corticotropin Releasing Factor (CFR) has been shown to be involved in the alteration of gastric motility induced by stress in animals (Gue et al., 1991; Monnikes et al., 1993). CRF has been proposed as a Central Nervous System mediator of the effects of stress and of acceleration of large bowel transit (Williams et al., 1987). There is evidence that CRF may influence gastrointestinal motility through the modulation of the parasympathetic nervous system; therefore an increase in central cholinergic activity is known to activate colonic motility (Boom et al., 1965).

In conclusion we have demonstrated the existence of specific antimicrobial resistant *E. coli* strains associated with certain intestinal segments (upper intestinal

tract). When animals are exposed to cold or heat stress, an increase in propulsion of intestinal contents occurs causing a reduction in transit time and an outflow of resistant *E. coli* moves from the upper to the lower tract and is shed into the environment.

LITERATURE CITED

- Allison, M. J., I. M. Robinson, J. A. Bucklin, and G. D. Booth.** 1979. Comparison of bacterial populations of the pig cecum and colon based upon enumeration with specific energy sources. *Appl. Environ. Microbiol.* **37**: 1141-1151.
- Aserkoff, B., and J. B. Bennett.** 1969. Effect on antibiotic therapy in acute salmonellosis in the fecal excretion of salmonellae. *N. Engl. J. Med.* **281**: 636-640.
- Boom, R., G. Chavez-Ibarra, J. J. Del Villar, and R. Hernandez-Pron.** 1965. Changes of colonic motility induced by electrical and chemical stimulation of the forebrain and hypothalamus in cats. *Int. J. Neuropharmacol.* **4**: 169-175.
- Brain, P. F.** 1971. Mammalian behavior and the adrenal cortex-a review. *Behav. Biol.* **7**: 453-477.
- Clewell, D. B., and C. G. Burke.** 1986. Conjugative transposons and the dissemination of antibiotic resistance in streptococci. *Ann. Rev. Microbiol.* **40**: 635-659.
- Cohen, S.** 1976. Transposable genetic elements and plasmid evolution. *Nature* **263**: 731-738.
- Corrier, D. E., C. W. Purdy, and J. R. DeLoach.** 1990. Effects of marketing stress on fecal excretion of *Salmonella* spp in feeder calves. *Am. J. Vet. Res.* **51**: 866-869.
- Davies, J., and J. Anandan.** 1970. The evolution of R factors: a study of the pre-antibiotic community in Borneo. *N. Engl. J. Med.* **282**: 117-122.
- Davies, J.** 1979. General mechanisms of antimicrobial resistance. *Rev. Infect. Dis.* **1**: 23-29.
- Davies, J., and D. L. Smith.** 1978. Plasmid-determined resistance to antimicrobial agents. *Ann. Rev. Microbiol.* **32**: 469-518.
- Dawson K. L., B. E. Langlois, T. S. Stahly, and G. L. Cromwell.** 1984. Antibiotic Resistance in anaerobic and coliform bacteria from the intestinal tract of swine fed therapeutic and subtherapeutic concentration of chlortetracycline. *J. Anim. Sci.* **58**: 123-131.
- Dupont, H. L., and J. H. Steele.** 1987. Use of antimicrobial agents in animal feeds: implications for human health. *Rev. Infect. Dis.* **9**: 447-460.

Dvorak, M. 1971. Adrenocortical function in the hypoglycemia of newborn pigs. *Acta Vet. Brno.* **2**: 25-27.

Falkow, S. 1975. The prevalence and ecology of R-factors, p. 58-269. *In* Infection multiple drug resistance. Pion Ltd., London.

Finland, M. 1972. Changing patterns of susceptibility of common bacterial pathogens to antimicrobial agents. *Ann. Intern. Med.* **76**: 1009-1036.

Finland, M. 1979. Emergence of antibiotic resistance in hospitals, 1935-1975. *Rev. Infect. Dis.* **1**: 4-21.

Frost, A. J. 1991. Antibiotics and animal production, p. 181-194. *In* J. B. Woolcock (ed.), Microbiology of animals and animal products, World Animal Science. Elsevier Science Publishers B. V., Amsterdam.

Garrick, T., F. W. Leung, S. Buack, K. Hirabayashik, and P. H. Guth. 1986. Gastric motility is stimulated but overall flow is unaffected during cold restraint in the rat. *Gastroenterology* **91**: 141-148.

Goldsmith, D. P. J. 1973. Changes in desquamation rate of jejunal epithelium in cats during fasting. *Digestion* **8**: 130-141.

Komal, M., and S. Kimura. 1979. Effects of restricted diet and intestinal flora on the lifespan of small intestine epithelial cells in mice. *J. Nutr. Sci. Vitaminol.* **25**: 87-94.

Gracey, M., S. Suharjono, and D. E. Stone. 1973. Microbial contamination of the gut: another feature of malnutrition. *Am. J. Clin. Nutr.* **26**: 1170-1174.

Grubb, J. A., and B. A. Dehority. 1975. Effects of an abrupt change in ration from all roughage to high concentrate upon rumen microbial numbers in sheep. *Appl. Microbol.* **30**: 404-412.

Gue, M., J. L. Junien, and L. Bueno. 1991. Conditioned emotional response in rats enhances colonic motility through the central release of corticotropin-releasing factor. *Gastroenterology* **100**: 964-970.

Guinee, P.A. 1972. Bacterial drug resistance in animals, p 95-104. *In* V. Kremery, L. Rosival, and T. Watanabe (ed.), Bacterial plasmids and antibiotic resistance. Springer-Verlag, New York.

Hartley, C. L., and M. H. Richmond. 1975. Antibiotic resistance and survival of *E. coli* in the alimentary tract. *Br. Med. J.* **4**: 71-74.

Heyworth, B., and J. Brown. 1975. Jejunal microflora in malnourished gambian children. *Arch. Dis. Child.* **50**: 27-33.

Hinton, M., A. H. Linton, and A. J. Hedges. 1985. The ecology of *Escherichia coli* in calves reared as dairy-cow replacements. *J. Appl. Bacteriol.* **58**: 131-138.

Hinton, M., D. J. Hampson, E. Hampson, and A. H. Linton. 1985. A comparison of the ecology of *Escherichia coli* in the intestine of healthy unweaned pigs and pigs after weaning. *J. Appl. Bacteriol.* **58**: 471-478.

Hinton, M. 1986. The ecology of *E. coli* in animals including man with particular reference to drug resistance. *Vet. Rec.* **119**: 420-426.

Hinton, M., V. Allen, and A. H. Linton. 1982. The biotyping of *Escherichia coli* isolated from healthy farm animals. *J. Hyg.* **88**: 543-555.

Hinton, M., A. J. Hedges, and A. H. Linton. 1985. The ecology of *Escherichia coli* in market calves fed a milk-substitute diet. *J. Appl. Bacteriol.* **58**: 27-35.

Hirsh, D. C., and N. Wiger. 1978. The effect of tetracycline upon the spread of bacterial resistance from calves to man. *J. Anim. Sci.* **46**: 1437-1446.

Hirsh, D. C., and N. Wiger. 1977. Effect of tetracycline upon transfer of an R plasmid from calves to human beings. *Am. J. Vet. Res.* **38**: 1137-1139.

Hirsh, D. C., G. C. Burton, and D. C. Blendon. 1974. The effect of tetracycline upon establishment of *Escherichia coli* of bovine origin in the enteric tract of man. *J. Appl. Bacteriol.* **37**: 327-333.

Holdeman, L. V., I. J. Good, and W. E. Moore. 1976. Human fecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress. *Appl. Environ. Microbiol.* **31**: 359-375.

Hummel, R., H. Tschape, and W. Whitte. 1986. Spread of plasmid-mediated nourseothricin resistance due to antibiotic use in animal husbandry. *J. Basic Microbiol.* **26**: 461-466.

Hungate, R. E. 1966. The rumen and its microbes. Academic Press, New York.

Institute of Medicine. 1988. Human health risks with the subtherapeutic use of penicillin or tetracyclines in animal feed. Committee on human health risk assessment of using subtherapeutic antibiotics in animal feeds. National Academy Press, Washington, D. C.

Kilgour, R., and H. de Langen. 1970. Stress in sheep resulting from management practices. *Proc. N. Z. Soc. Anim. Prod.* **30**: 65-76.

Kreuzer, K. N. 1992. Genetic variation and exchange, p. 125-152. *In* W.K. Joklik, H. P. Willett, D. B. Amos, and C. M. Wilfert (ed.), *Zinsser Microbiology*, 20th ed. Appleton & Lange, Norwalk.

Lacey, R. W. 1975. Antibiotic resistance plasmids of *Staphylococcus aureus* and their clinical importance. *Bacteriol. Rev.* **39**: 1-32.

Langlois, B. E., K. A. Dawson, G. L. Cromwell, and T. S. Stahly. 1986. Antibiotic resistance in pigs following a 13 year ban. *J. Anim. Sci.* **62**: 18-32 (Suppl. 3).

Langlois, B. E., K. A. Dawson, T. S. Stahly, and G. L. Cromwell. 1984. Antibiotic resistance of fecal coliforms from swine fed subtherapeutic and therapeutic levels of chlortetracycline. 1984. *J. Anim. Sci.* **58**: 666-674.

Lee, A., and E. Gemmel. 1972. Changes in the mouse intestinal microflora during weaning: role of volatile fatty acids. *Infect. Immun.* **5**: 1-7.

Lenz H. J., A. Raedler, H. Greten, W. W. Vale, and J. E. Rivler. 1988. Stress-induced gastrointestinal secretory and motor responses in rats are mediated by endogenous corticotropin-releasing factor. *Gastroenterology* **95**: 1510-1517.

Levy, S. B. 1992. The antibiotic paradox: how miracle drugs are destroying the miracle. Plenum Press. New York.

Levy, S. B., and B. M. Marshall. 1980. Genetic transfer in the natural environment, p. 61-76. *In* M. Sussman, C. H. Collin, S. K. Skinner, and D. E. StewardTull (ed.), *The release of genetically engineered microorganisms*. Academic Press, London.

Levy, S. B., G. B. FitzGerald, and A. B. Macone. 1976. Changes in intestinal flora of farm personnel after introduction of a tetracycline-supplemented feed on a farm. *N. Engl. J. Med.* **295**: 583-588.

Levy, S. B. 1978. Emergence of antibiotic-resistant bacteria in the intestinal flora of farm inhabitants. *J. Infect. Dis.* **137**: 688-690.

Linton, A. H., P. A. Lee, M. H. Richmond, W. A. Gillespie, A. J. Rowland, and V. N. Baker. 1972. Antibiotic resistance and transmissible R-factors in the intestinal coliform flora of healthy adults and children in an urban and a rural community. *J. Hyg.* **70**: 99-104.

Linton, A. H., K. Howe, and A. D. Osborne. 1975. The effects of feeding tetracycline, nitrovin and quindoxin on the drug resistance of coll-aerogenes bacteria from calves and pigs. *J. Appl. Bacteriol.* **38**: 255-275.

Marshall, B., D. Petrowski, and S. B. Levy. 1990. Inter-and intraspecies spread of *Escherichia coli* in a farm environment in the absence of antibiotic usage. *Proc. Natl. Acad. Sci. USA* **87**: 6609-6613.

Marsik, F. J., J. T. Parisi, and D. C. Blendon. 1975. Transmissible drug resistance of *Escherichia coli* and *Salmonella* from humans, animals, and their rural environments. *J. Infect. Dis.* **132**: 296-302.

Mata, L. J., F. Jimenez, M. Cordon, R. Rosales, E. Prera, R. E. Schneider, and F. Viteri. 1972. Gastrointestinal flora of children with protein-calorie malnutrition. *Am. J. Clin. Nutr.* **25**: 1118-1126.

Mollitoris, E, D. J. Fagerberg, C. L. Quarles, and M. I. Krichevsky. 1987. Changes in antimicrobial resistance in fecal bacteria associated with pig transit and holding times at slaughter plants. *Appl. Environ. Microbiol.* **53**: 1307-1310.

Monnikes, H., G. Schmidt, and Y. Tache. 1993. Psychological stress-induced accelerated colonic transit in rats involves hypothalamic corticotropin-releasing factor. *Gastroenterology* **104**: 716-723.

Moon, H. W., P. Y. Fung, R. E. Isaacson, and G. D. Booth. 1979. Effects of age, ambient temperature, and heat-stable *Escherichia coli* enterotoxin on intestinal transit in infant mice. *Infect. Immun.* **25**: 127-132.

Morishita, Y., and M. Ogata. 1970. Studies on the alimentary flora of pigs. V. Influence of starvation on the microbial flora. *Jap. J. Vet. Sci.* **32**: 19-24.

News, H. C., C. E. Cherubin, E. D. Longo, B. Flouton, and J. Winter. 1975. Antimicrobial resistance and R-factor transfer among isolates of salmonellae in northeastern United States: a comparison of human and animal isolates. *J. Infect. Dis.* **132**: 617-622.

O'Brien, T. F., J. F. Acar, A. A. Medeiros, R. A. Norton, F. Goldstein, and R. L. Kent. 1978. International comparison of prevalence of resistance to antibiotics. *J. Am. Med. Assoc.* **239**: 1518-1523.

O'Brien, T. F., R. A. Norton, R. L. Kent, and A. A. Medeiros. 1977. International surveillance of prevalence of antibiotic resistance. *J. Antimicrob. Chemother.* **3 (Suppl. C)**: 59-66.

Odelson, D. A., J. L. Rasmussen, C. J. Smith, and F. L. Macrina. 1987. Extrachromosomal systems and gene transmission in anaerobic bacteria. *Plasmid* **17**: 87-109.

Petrocheillou, V., and M. H. Richmond. 1976. Distribution of R plasmids among the O-antigen types of *Escherichia coli* isolated from various clinical sources. *Antimicrob. Agents Chemother.* **9**: 1-5.

Petrocheillou, V., M. H. Richmond, and P. M. Bennett. 1977. Spread of a single plasmid clone to an untreated individual from a person receiving prolonged tetracycline therapy. *Antimicrob. Agents Chemother.* **12**: 219-225.

Petrocheillou, V., J. Grinsted, and M. H. Richmond. 1976. R-plasmid transfer in vivo in the absence of antibiotic selection pressure. *Antimicrob. Agents Chemother.* **10**: 753-761.

Saffran, M. 1962. Mechanisms of adrenocortical control. *Br. Med. Bull.* **18**: 122-126.

Saunders, J. R. 1975. Transposable resistance genes. *Nature* **258**: 384.

Savage, D. C. 1982. The effects of stress, diet and environment on the stability of the gastro-intestinal microflora, p. 23-31. *In*: G. E. White (ed.), Normal and induced changes in the gastro-intestinal microflora in man and animals with special regard to animal performance. International symposium, Oslo, September 25/26, 1980. Verlag Paul Parey, Berlin and Hamburg.

Savage, D. C. 1977. Microbial ecology of the gastrointestinal tract. *Ann. Rev. Microbiol.* **31**: 107-133.

Schaedler, R. W., R. Dubos, and R. Costello. 1965. The development of the bacterial flora in the gastrointestinal tract on mice. *J. Exp. Med.* **122**: 59-66.

Siegel, D., W. G. Huber, and S. Drysdale. 1975. Human therapeutic and agricultural uses of antibacterial drugs and resistance of the enteric flora of humans. *Antimicrob. Agents Chemother.* **8**: 538-543.

Smith, H. W. 1969. Transfer of antibiotic resistance from animal and human strains of *Escherichia coli* to resident *E. coli* in the alimentary tract of man. *Lancet* **1**: 1174-1176.

Smith, H. W. 1965. Observation on the flora of the alimentary tract of animals and factors affecting its composition. *J. Pathol. Bacteriol.* **89**: 95-122.

Smith, J. C., S. M. Markowitz, and F. L. Macrina. 1981. Transferable tetracycline resistance in *Clostridium difficile*. *Antimicrob. Agents Chemother.* **19**: 997-1003.

Sogaard, H. 1973. Incidence of drug resistance and transmissible R factors in strains of *E. coli* isolated from faeces of healthy pigs. *Acta Vet. Scand.* **14**: 381-391.

Solomons, I. A. 1978. Antibiotics in animal feeds-human and animal safety issues. *J. Ani. Sci.* **46**: 1360-1368.

Stanghellini, V., J. R. Malagelada, A. R. Zinsmeister, V. L. Go, and P. C. Kao. 1983. Stress-induced gastroduodenal motor disturbances in humans: possible humoral mechanisms. *Gastroenterology* **85**: 83-91.

Stephen, A. M., H. S. Wiggins, and J. H. Cummings. 1987. Effect of changing transit time on colonic microbial metabolism in man. *Gut* **28**: 60-609.

Tally, F. P., and M. H. Malamy. 1986. Resistance factors in anaerobic bacteria. *Scand. J. Infect. Dis.* **49 (Suppl)**: 56-63.

Tannock, G. W., and D. C. Savage. 1974. Influences of dietary and environmental stress on microbial populations in the murine gastrointestinal tract. *Infect. Immun.* **9**: 591-598.

Tannock, G. W. 1983. Effect of dietary and environmental stress on the gastrointestinal microbiota, p. 517-539. *In*: D. J. Hentges (ed.), *Human intestinal microflora in health and disease*. Academic Press, New York.

Tenover, F. C. 1986. Minireview: Studies on antimicrobial resistance genes using DNA probes. *Antimicrob. Agents Chemother.* **29**: 721-725.

Threlfall, E. J., L. R. Ward, and B. Rowe. 1978. Epidemic spread of a chloramphenicol-resistant strain of *Salmonella typhimurium* phage type 204 in bovine animals in Britain. *Vet. Rec.* **103**: 438-440.

Timoney, J. F. 1981. R plasmids in pathogenic enterobacteriaceae from calves, p. 547-555. *In* S. B. Levy, R. C. Clowes, and E. L. Koenig (ed.), *Molecular biology, pathogenicity, and ecology of bacterial plasmids*. Plenum Press, New York.

Valori, R. M., D. Kumar, and D. L. Wingate. 1986. Effects of different types of stress and of prokinetic drugs on the control of the fasting motor complex in humans. *Gastroenterology* **90**: 890-900.

Van Houweling C. D., and J. H. Galner. 1978. Public health concerns relative to the use of subtherapeutic levels of antibiotics in animal feeds. *J. Anim. Sci.* **46**: 1413-1424.

Varel, V. H., W. G. Pond, J. C. Pekas, and J. T. Yen. 1982. Influence of high-fiber diet on bacterial populations in intestinal tracts of obese and lean genotype pigs. *Appl. Environ. Microbiol.* **44**: 107-112.

Visek, W. J. 1978. Mode of growth promotion by antibiotics. *J. Ani. Sci.* **46**: 1447-1469.

Walton, J. R. 1983. Modes of action of growth promoting agents. *Vet. Res. Commun.* **7**: 1-7.

Williams, L. P., K. W. Newell. 1970. Salmonella excretion in joy-riding pigs. *Am. J. Public Health.* **60**: 926-929.

Williams, C. L., R. G. Villar, J. M. Peterson, and T. F. Burks. 1988. Stress-induced changes in intestinal transit in the rat: a model for irritable bowel syndrome. *Gastroenterology* **94**: 611-621.

Williams, C. L., J. M. Peterson, R. G. Villar, and T. F. Burks. 1987. Corticotropin releasing factor directly mediates colonic responses to stress. *Am. J. Physiol.* **253**: G582-586.

Yates, F. E., and J. Urquhart. 1962. Control of plasma concentrations of adrenocortical hormones. *Physiol. Rev.* **42**: 359-429.

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